Cancer Drug Discovery and Development

Robert Clarke *Editor*

The Unfolded Protein Response in Cancer

Humana Press

Cancer Drug Discovery and Development

Series Editor
Beverly A. Teicher
Bethesda, MD, USA

The Cancer Drug Discovery and Development series (Beverly A Teicher, series editor) is the definitive book series in cancer research and oncology, providing comprehensive coverage of specific topics and the field. Volumes cover the process of drug discovery, preclinical models in cancer research, specific drug target groups and experimental and approved therapeutic agents. The volumes are current and timely, anticipating areas where experimental agents are reaching FDA approval. Each volume is edited by an expert in the field covered and chapters are authored by renowned scientists and physicians in their fields of interest.

More information about this series at http://www.springer.com/series/7625

Robert Clarke
Editor

The Unfolded Protein Response in Cancer

Humana Press

Editor
Robert Clarke
Department of Oncology
Georgetown University Medical Center
Washington, DC, USA

ISSN 2196-9906 ISSN 2196-9914 (electronic)
Cancer Drug Discovery and Development
ISBN 978-3-030-05065-8 ISBN 978-3-030-05067-2 (eBook)
https://doi.org/10.1007/978-3-030-05067-2

Library of Congress Control Number: 2018968116

This Humana Press imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

The unfolded protein response (UPR) is an ancient (in the evolutionary sense) stress response network, components of which are readily detected in both prokaryotes and eukaryotes. There continue to be major advances in our understanding of the UPR and its ability to integrate multiple cellular functions including key aspects of basic cellular metabolism, autophagy, and apoptosis. Nonetheless, much remains unclear, and several key components of the UPR can be regulated outside the full activation of the three primary response arms regulated by IRE1α, PERK, and ATF6. This volume describes current knowledge of each of the arms of the UPR, how their activation/repression are regulated, integrated, and coordinated, how UPR components affect cancer cell biology and responsiveness to therapeutic interventions, and how UPR components/activities offer potentially novel targets for drug discovery, repurposing, and development. Autophagy and the UPR are intimately related and they can affect the pathobiology of diseases other than cancer. Hence, this book also includes a chapter on neurodegenerative diseases by way of introduction to these activities. The primary goal of this volume is to provide a broad overview of the UPR, guided by the most recent information, with specific examples of how the UPR and/or specific components of its signaling contribute to cancer biology. This book also addresses the role of the UPR in affecting the responsiveness of cancer cells to systemic endocrine and chemotherapeutic interventions, representing unique features of this volume relative to other works in the field. Each chapter in this book is written so that it can be read and understood independently. While some topics are covered more than once, the reader can choose among the chapters of greatest relevance or read the book in its entirety. The general nature of this book will be useful to researchers and students with an interest in understanding the role of the UPR in affecting cancer cell fate, with important insights from cellular contexts outside cancer as provided in the chapters on development and neurodegenerative diseases. The Editor wishes to thank Ms. Jennifer Kluge for her many contributions in the preparation of this book.

Washington, DC, USA — Robert Clarke

Contents

Contributors

Joseph E. Chambers Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

Xi Chen Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA

Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA

Robert Clarke Department of Oncology, Georgetown University Medical Center, Washington, DC, USA

Katherine L. Cook Department of Surgery and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Caia S. Dominicus Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

The Francis Crick Institute, London, UK

Adrian L. Harris Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK

Feng Hong Department of Microbiology and Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA

Rong Hu Department of Oncology, Georgetown Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA

V. Craig Jordan Department of Breast Medical Oncology, University of Texas MD Anderson Cancer Center, Unit 1354, Houston, TX, USA

Zihai Li Department of Microbiology and Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA

Elke Malzer Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

MRC Harwell Institute, Oxfordshire, England, UK

Stefan J. Marciniak Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

Jordan Morreall Department of Microbiology and Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA

Charbel Moussa Laboratory for Dementia and Parkinsonism, Translational Neurotherapeutics Program, Department of Neurology, Georgetown University Medical Center, Washington, DC, USA

Vruti Patel Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

University College London, London, UK

Fanglue Peng Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA

Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA

Surojeet Sengupta Department of Oncology, Georgetown Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA

Dean C. Singleton Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand

Na Zhao Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA

Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA

About the Editor

Robert Clarke, PhD, DSc, FRSBiol, FRSChem, is Professor of Oncology at Georgetown University (Washington, DC, USA). He is Dean for Research at Georgetown University Medical Center (GUMC), home to over 80% of sponsored research at Georgetown University. Dr. Clarke is also Director of the Biomedical Graduate Research Organization, one of four academic units within GUMC. A member of the Georgetown-Lombardi Comprehensive Cancer Center, he is currently Co-Leader of the Breast Cancer Program.

Dr. Clarke earned a DSc in 1999, a PhD in 1986, and a MSc in 1982 (each in Biochemistry) from the Queen's University of Belfast, Northern Ireland, and a BSc (Biological Sciences) in 1980 from the University of Ulster, Northern Ireland. He completed his postdoctoral training as a Breast Cancer Study Group Fellow with Dr. Marc E. Lippman at the National Cancer Institute (National Institutes of Health, Bethesda, MD, USA). In 1989, Dr. Clarke joined the Department of Physiology and Biophysics at Georgetown University. He served as Secretary/Treasurer of the Georgetown University Faculty Senate from 2004 to 2007.

Dr. Clarke is an elected Fellow of the Royal Society of Chemistry (UK), the Royal Society of Medicine (UK), and the Royal Society of Biology (UK). An internationally recognized leader in breast cancer research, he was honored among the 100 most frequently published breast cancer researchers of the twentieth century at the 23rd San Antonio Breast Cancer Symposium (2000). Dr. Clarke completed full terms as chair of both the National Institutes of Health grant peer-review study sections National Center for Complementary and Alternative Medicine: *Basic Science* (2002–2008) and National Cancer Institute: *Basic Mechanisms of Cancer Therapeutics* (2011–2013), and he has chaired several NIH Special Emphasis Panels and grant review panels for the US Army Medical Research and Materiel Command (*Breast Cancer Research Program*). Regularly invited to speak about his research, he completed a 3-year term as the *NCI-SigmaXi Distinguished Lecturer* in 2014. Currently, Dr. Clarke is a Senior Editor for the journal *Cancer Research*, and he serves on the editorial boards of over a dozen international scientific journals.

Chapter 1
Introduction: The Unfolded Protein Response

Robert Clarke

Abstract The translation and appropriate folding of proteins is critical for the maintenance of cellular function. This process is tightly controlled, and it can create a significant energy demand, particularly in secretory cells. Inadequate folding of proteins, as may occur with an insufficient energy supply, can cause unfolded, misfolded, or damaged proteins to accumulate in the endoplasmic reticulum. The consequent endoplasmic reticulum stress leads to activation of the unfolded protein response (UPR). In stressed mitochondria, a similar process is activated (mitochondrial unfolded protein response). The unfolded protein response is an ancient stress response that coordinates multiple functions in an attempt to restore metabolic homeostasis. In higher organisms, three signaling arms, driven respectively by PERK, ATF6, and IRE1α, may be activated. Together, the signaling from these arms coordinates specific cellular functions including autophagy, cell metabolism, and apoptosis. From a cell fate perspective, the outcome of activating the unfolded protein response can be either the restoration of homeostasis and normal cell function, or the failure to do so leading to aberrant cellular function (including neoplastic transformation) and/or the eventual initiation of an irreversible programmed cell death. Hence, activation of the unfolded proteins response can be either prosurvival or prodeath. This book covers many aspects of the unfolded protein response, from its roles in normal cell development and some aspects of immunity, through to those associated with neoplastic transformation and drug resistance in cancer. Also included is a chapter on the role of UPR-activated autophagy in specific neurodegenerative disorders. The primary focus of these chapters is the unfolded protein response as activated by an endoplasmic reticulum stress. While each chapter may be read independently, the reader will gain a much broader perspective of the critical roles of the unfolded protein response when the chapters are read collectively.

R. Clarke (✉)
Department of Oncology, Georgetown University Medical Center, Washington, DC, USA
e-mail: clarker@georgetown.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_1

Keywords Autophagy · ATF4 · ATF6 · Endoplasmic reticulum stress · Cancer · Canonical signaling · Diabetes · eIF2α · GRP78 · Hematopoietic · IRE1α · Metabolism · Neurodegenerative · PERK · Protein synthesis · Ribosome · Translation · Transcription

Introduction

Living cells must synthesize the proteins that perform the functions that sustain their life. These proteins often require folding into a specific conformation(s) that enables them to act. Cells expend a significant amount of their available energy in executing the process of protein folding. Unfolded, misfolded, or damaged proteins must be eliminated because the accumulation of these proteins within the endoplasmic reticulum (EnR) causes the EnR to swell, become stressed (EnR stress), and fail to function optimally. A similar process and response can occur within mitochondria [1]. To address the challenges that this proteotoxic stress creates for cell function, EnR stress can activate a process that identifies and targets unfolded proteins for degradation. The process that is activated is called the unfolded protein response (UPR); in mitochondria, this response is called the mitochondrial UPR (UPRmt) [2].

Since the requirements to fold proteins properly and to eliminate unfolded or damaged proteins are fundamental to life, features of the UPR likely arose relatively early during evolution. For example, components of UPR action are readily detected in species as diverse as yeast and human. The UPR network has three signaling arms regulated, respectively, by protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 alpha (IRE1α). Each arm of the three UPR may have evolved over different timeframes. These is some controversy over which is the oldest arm, with the IRE1α arm often being considered the older. However, orthologs of IRE1α are rarely seen in protozoans, where activities similar to PERK signaling are more often detected [3].

In higher eukaryotes, the UPR can help the cell respond to stresses that include the limitations in cellular energy and oxygen that are of particular relevance to cancer cells growing in poorly vascularized solid tumor microenvironments. For example, the instruction to degrade unfolded or damaged proteins releases their amino acids and other components, including sugars and fatty acids, for reuse within the cells. This recycling can save energy by supporting intermediate metabolism within the cell [4]. Moreover, activation of the UPR, for example as induced following EnR stress, integrates the signaling that controls multiple cellular functions with the goal of enabling cell survival or executing a programmed cell death.

In its canonical signaling representations (Fig. 1.1), the three signaling arms of the UPR network are each regulated by the common upstream activator glucose-regulated protein-78 (GRP78), also known as binding immunoglobulin protein (BiP) or heat shock protein A5 (HSPA5; Human Genome Organization symbol for this gene). GRP78, a generally short-lived, EnR membrane-resident protein [5] is one of a series of molecular chaperones that bind to unfolded or misfolded proteins accumulating in the lumen of stressed EnR, and then targets these proteins for

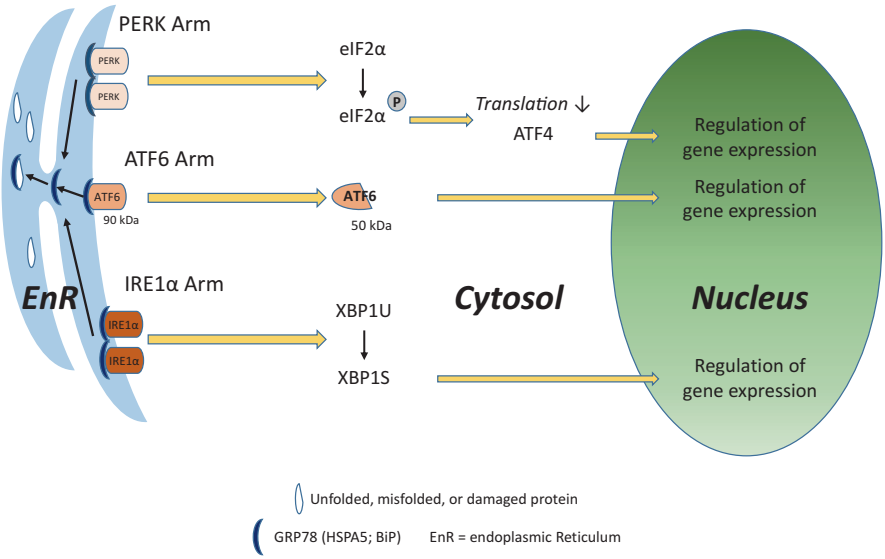

Fig. 1.1 Simple representation of the structure of the canonical unfolded protein response (UPR) network. Upon activation, GRP78 (HSPA5, BiP) is released from each of the three sensors (PERK, ATF6, IRE1α) to act as a chaperone for unfolded, misfolded, or damaged proteins. Each sensor is activated upon release from GRP78 to initiate signaling in its respective UPR arm. Activated PERK controls eIF2α phosphorylation that can then inhibit translation and activate ATF4. ATF6 (90 kDa) is cleaved by site 1 and site 2 proteases to release a transcriptionally active cleaved ATF6 (50 kDa). Activated IRE1α removes a 26 bp fragment from unspliced XBP1 (XBP1U) creating a frame shift that encodes for a spliced XBP1 (XBP1S) that can now act as a nuclear transcription factor

refolding or degradation. Unfolded, misfolded, or damaged proteins generally have features exposed on their surface that are recognized by chaperone proteins as being inappropriate. These features include cysteine residues that would normally be paired, hydrophobic regions that are often internal rather than on a protein's surface, and/or immature glycans. Chaperone binding prevents unfolded proteins from aggregating and assists in their refolding or retrotranslocation back out of the EnR or mitochondria to the cytosol for degradation. When acting as a chaperone, GRP78 binding to misfolded proteins favors degradation in part by recognizing existing ubiquitin moieties and catalyzing additional ubiquitination [6]. Degradation of marked proteins involves the ubiquitin-proteasome system [7, 8]. An EnR-activated autophagy (ERAA) pathway also can be activated as a means to degrade unfolded proteins [9].

GRP78 is normally bound to three EnR membrane-resident proteins (PERK, ATF6, and IRE1α), which are consequently maintained in an inactive state. When released from GRP78, which leaves to fulfill its molecular chaperone role in response to an EnR stress, PERK, ATF6, and IRE1α can regulate their respective signaling cascades. Unresolved UPR activation could lead to prolonged repression of transcription and translation, and to excessive autophagic degradation of critical cellular components, that can induce an irreversible cell death cascade [10]. Hence,

feedback inhibition within the UPR can normally return signaling to its resting state once the stress has been resolved. The complexity of these and other control mechanisms within the UPR is still being defined. Perhaps the feedback control that has been most widely studied is that controlling the regulation of eukaryotic initiation factor 2-alpha (eIF2α) phosphorylation in the PERK arm of the UPR. eIF2α can control the rate of protein translation. When dephosphorylated by PP1, as regulated by activating transcription factor 4 (ATF4) and growth arrest and DNA damage-inducible protein-34 (GADD34; PPP1R15A), the eIF2α-driven inhibition of translation is relieved and the rate of translation can return to normal.

The importance of the UPR in managing protein load has been studied in both normal and diseased tissues, with key fundamental work evident in the pancreas with respect to insulin production in the normal and diabetic states [11, 12] and in hematopoietic cells [13, 14]. The importance of regulating unfolded proteins in prion diseases has recently begun to attract notable attention. Inappropriate protein aggregation is particularly problematic in the brain and contributes to several major neurological disorders [15]. The need to ensure appropriate control of protein production and folding is likely to be different in tissues that secrete large amounts of proteins when compared with those that are non-secretory and/or not proliferating.

In several cancers and other diseases, UPR components can be regulated differently from normal tissues. The control of cell proliferation and survival as determined by estrogen receptor alpha (ER; ESR1) activation in many breast cancers has major implications for cell fate determination [16]. The importance of endocrine regulation of the UPR, and loss of this regulation when cells become resistant to drugs that target ER activity, has been established [4, 10, 16–18]. In this example, aspects of canonical UPR signaling are evident in cancer cells. However, the control of individual features of canonical signaling can be modified by hormones, growth factors, or other changes in cells including those affected by other signals received from the tumor microenvironment. Together, these intrinsic and extrinsic activities contribute significantly to cell context-specific modifications to UPR regulation and execution within an individual cancer cell.

The timing of activation of each UPR arm, and the relative importance of each arm once activated, remains unclear and likely differs by cell context and the nature and/or potency of the stressor(s). UPR components activated differentially in duration or level of activity likely also contribute to whether the ultimate cell fate outcome is death versus survival and/or proliferation. Some cells can activate an anticipatory UPR, including estrogen responsive breast cancer cells [18]. Translational inhibitory activities often occur first and within minutes of UPR activation [19]. Blocking the translation of existing mRNA templates would have an immediate effect on the number of protein molecules requiring folding and produce a rapid decline in the energy needs within the cell. The speed of activation implies that the executors of this inhibition are already present and do not require transcription or translation. Inhibition of transcription occurs later, often concurrent with chromatin remodeling [19]. This sequencing allows for the general inhibition of mRNA and protein production and the preferential synthesis of any new proteins required (including molecular chaperones) and (ideally) only in the amounts the cell

can manage. Concurrent chromatin remodeling would allow the cells to retain this pattern of expression for prolonged periods and possibly dampen the potential for the transcriptional regulation from UPR activation to cycle on and off inappropriately. For any cell already committed to completing a turn of the cell cycle, its progeny also would arise programmed to manage their protein production in a stressed environment, likely also reflecting any cell context-specific modifications.

A key consequence of UPR activation is its direction to degrade unfolded proteins through an EnR-associated degradation (ERAD) [20, 21]. For example, misfolded glycoproteins are managed by genes in the EDEM family (EnR degradation-enhancing α-mannosidase-like protein). This process targets the misfolded proteins for eventual degradation by the ubiquitin-proteasome [22]. Proteins bound to chaperones are also removed through chaperone-mediated autophagy; other proteins targeted for degradation may be collected from the cytosol through microautophagy [23, 24]. Macroautophagy can also be induced by UPR signaling [4]. It is through the degradation of unfolded or damaged proteins, or damaged or excess organelles, that the products so released can be used as substrates for intermediate metabolism to help restore metabolic homeostasis. Hence, signaling through the UPR network to control autophagy and cellular metabolism represents an example of the functional integration required to optimize the ability of a cell to respond to stress.

Within the UPR network, the signaling arms that are perhaps the most widely studied for their impact on cell fate are those for PERK and IRE1α. While the control of transcriptional responses to stress are complex [19], activation of the PERK signaling arm can essentially modify the rates of transcription, translation, and protein transport into the EnR to help align the resources available with the protein folding load and so restore homeostasis. This arm can also initiate cell death signaling through activation of C/EBP homologous protein (CHOP; DDIT3). Conversely, activation of IRE1α leads to the unconventional splicing of X-box binding protein-1 (XBP1), which can drive prosurvival signaling through XBP1 regulation of select members of the BCL2 family and the coordinated regulation of autophagy and apoptosis [4, 10]. The balance of prodeath and prosurvival signaling is a major determinant of cell fate in normal and neoplastic cells. In this regard, the altered regulation of UPR signaling appears to be central to the process of neoplastic transformation and cancer cell survival [25], in addition to representing a fundamental component of cancer cells' adaptive responses to the cellular stresses induced by many systemic anticancer therapies. Thus, the UPR likely also contributes to de novo and/or acquired resistance to some anticancer drugs.

A Brief Overview of This Book

The various activities of the UPR noted above are described and discussed in more detail throughout this book. Starting with the role of UPR in development and ending with a description of its regulation of autophagy and the consequences thereof

for neuronal cell fate in neurodegenerative diseases, UPR network signaling and function are presented from multiple different perspectives. Each chapter in this book is written so that it can be read and understood independently, without necessarily referring to other chapters. While this leaves some ground covered more than once, this approach achieves two important goals. Firstly, it presents the UPR in different contexts while underscoring the central nature of this ancient response in cancer biology. Secondly, because each chapter can stand alone, the reader can choose the chapters of greatest relevance or interest without the need to read the book in its entirety. While it is not possible to cover all aspects of the UPR in normal development and in disease, the chapters in this book provide readers with insight from several perspectives into the importance of this stress response network. Moreover, components of the UPR that can affect other functions independent of the need to ameliorate multiple different forms of cellular stress are also considered.

The chapter by Dominicus et al. [26] addresses the role of EnR stress and the UPR in the context of development. Each of the major control components of the UPR is introduced (PERK, IRE1α, ATF6), providing an excellent overview of the canonical processes attributed to UPR signaling. In the context of development, the authors explore the role of the IRE1-XBP1 arm in embryonic liver and placenta, pancreas and stomach, B-cells and plasma cells, dendritic cells, osteogenesis, and in an epithelial-to-mesenchymal transition (EMT). The PERK-eIF2α-ATF4 arm is considered in the development of the pancreas, and the processes of osteogenesis and adipogenesis. While the ATF6 arm is perhaps the least well studied of the three UPR arms, its key role in the development of myoblasts, osteogenesis, and photoreceptor biology is presented. A short section also discusses the role of ATF6 paralogues in cellular development. Overall, it is now evident that the role of the UPR extends well beyond a canonical EnR stress response network for cells [27]. While it might be expected that these activities would be most evident in secretory cells, the UPR is also a key player in the development of non-secretory cells. This latter series of activities in non-secretory cells may be a broader reflection of the general need of all cells to maintain control over the use of their available resources to synthesize and fold the new proteins essential for the repair and replacement of damaged or aged organelles and to maintain basic cellular functions over time.

The roles of the UPR in the development of neoplasia and anticancer drug responsiveness are introduced in the chapter by Morreall et al. [28]. Following on from the observations from the development of normal cells in the prior chapter, these authors address the role of UPR signaling in enabling the maintenance of cell proliferation and neoplastic transformation, and of an EMT that has been widely implicated in the acquisition of a more aggressive, metastatic cancer phenotype. This chapter places the co-opting of the UPR at the center of these critical biological processes as they occur along the spectrum of changes that arise with the development of cancer, on through to enabling cancer cells to adapt to the stressors imposed by the systemic interventions applied during cancer treatment. As noted above, regulation of key components of the UPR can be altered in cancer cells. Examples include regulation of PERK activity by the oncogene MYC [29] and the requirement of MYC-transformed cells for XBP1 signaling to maintain growth [30]. MYC

is frequently dysregulated in breast cancer, where MYC can regulate the UPR and both glucose and glutamine uptake [31]. Here, Morreall et al. provide critical insights into how the UPR can regulate key aspects of cellular metabolism and cell fate, with their text organized respectively by the UPR arms of PERK, ATF6, and then IRE1α. Reflecting the complexity of signaling within the UPR, coordination among PERK, ATF6, and IRE1α is introduced briefly with clarity and specific examples. Following a similar organization, the chapter continues by discussing pharmacological interventions for each UPR arm, concluding with valuable insights into what the future may hold for anticancer treatments that could more effectively target the prosurvival signaling of the UPR. The hypoxia present in tumor microenvironments is also introduced here, and discussed in depth with a focus on drug resistance in the following chapter by Singleton and Harris.

Areas of hypoxia are common in solid tumors, reflecting their often poor and heterogeneous vascularization [32]. Since low oxygen levels can affect cellular metabolism and the oxidative environment of the EnR, reduced capacity for protein folding occurs leading to the accumulation of unfolded proteins and UPR activation [33]. Focusing on the role of ATF4, Singleton and Harris [34] provide a clear and detailed review of the role of hypoxia and UPR activation in conferring resistance to chemotherapy and radiotherapy. The authors begin with an overview of hypoxia and the molecular responses to a low oxygen environment, most notably the hypoxia inducible gene family (HIF) and HIF-related signaling. Activation of ATF4 also introduces one of the key integration points between the UPR and the cellular process of autophagy. Further integration between autophagy and UPR is described in the chapters by Clarke (on ER+ breast cancer) and Moussa (on neurodegenerative diseases). Here, Singleton and Harris describe how ATF4 signaling contributes to redox metabolism, amino acid homeostasis, angiogenesis, and cell invasion and metastasis. The role ascribed to ATF4 in this latter process reflects its role in regulating an EMT, a relationship introduced by Dominicus et al. earlier in this book. How ATF4 can alter responsiveness to both chemotherapy and radiotherapy is then presented, followed by some emerging approaches to targeting eIF2α, PERK, and ATF4. While in the relatively early phases of discovery, these approaches to targeting key features of UPR signaling could offer important new therapeutic interventions that could be of significant value to some cancer patients.

UPR signaling initiated by activation of PERK leads to phosphorylation of the eIF2α complex. The consequences of acute and chronic activation of eIF2α are compared in the chapter by Sengupta et al. [35]. The initial goal of eIF2α regulation is to inhibit global protein translation, thereby reducing the load of newly synthesized proteins for folding within the EnR. When considered in the context of the concurrent removal, degradation, and recycling of damaged or unfolded proteins, this effect can enable the cell to better align its available resources with the number of protein molecules that need appropriate folding. If homeostasis is restored, the cell will not induce a programmed cell death. In marked contrast, chronic activation of eIF2α-related signaling can lead to initiation and execution of apoptosis [36]. Appropriate regulation of this pathway is one of the critical control features of the UPR and can explain, in part, why activation of the UPR can result in either cell

survival or cell death. This chapter describes in detail how the PERK arm of the UPR regulates the protein translational machinery that plays a critical role in deciding a cell's fate following an EnR stress.

The next two chapters address the roles of XBP1 in breast cancer. The first of these chapters describes the roles of unspliced XBP1 (XBP1-U) and spliced (XBP1-S). The second of these chapters looks more closely at the role of XBP1 in triple negative breast cancers (TNBC), those that lack detectable expression of ER, progesterone receptor (PR), and the oncogene HER2. A later chapter by Clarke includes XBP1 activities within the broader role of the UPR as induced by endocrine therapies in ER+ breast cancer cells and their responsiveness to these agents.

As described by Hu and Clarke [37], unlike almost all other mRNAs that are spliced, XBP1 splicing occurs outside spliceosome assemblies. Rather, the endoribonuclease activity of IRE1α removes a 26 bp intron from within the unspliced XBP1 transcript. Splicing produces a frame shift that creates a template for translating an XBP1 protein now capable of acting as a transcription factor. XBP1 proteins arising from both the spliced (29 kDa) and unspliced transcripts (56 kDa) can be detected in human cancer cells. While the XBP1-S protein is more stable and has a longer half-life, XBP1-U can act as an endogenous dominant negative of XBP1-S. This chapter details the relative importance of both XBP1-U and XBP1-S, including a discussion of XBP1-S activities that occur outside the canonical UPR signaling. Indeed, several chapters include discussions of activities that can regulate key components of the UPR in a manner outside what is currently thought of as being canonical UPR signaling.

The second chapter on XBP1 focuses on another important breast cancer molecular subtype. Representing approximately 15% of all newly diagnosed breast cancers, TNBCs are often highly aggressive. Moreover, unlike the remaining two molecular subgroups (ER+ and/or PR+; HER2+) there is no standard molecular targeted therapy for TNBCs [38, 39]. In the chapter by Zhao et al. [40], the authors provide powerful new insights into the role of XBP1 as a central driver of the TNBC phenotype. An overview of the role of the UPR in normal mammary gland development provides critical background for the subsequent discussion of its role in TNBCs. Importantly, the authors link the UPR to hypoxia, nutrient deprivation, and MYC activity, extending the discussion to other important cancer-associated genes that are often overexpressed or mutated in breast and other cancers (RAS, PI3K, TP53). Some of these subjects were introduced briefly in earlier chapters, further reinforcing the central role of the UPR in cancer biology. This chapter further details the role of the UPR in cell communication within the tumor microenvironment and presents some exciting new therapies that could be developed by targeting features of the UPR often upregulated in cancer cells.

The most commonly diagnosed breast cancer subtype is comprised of tumors that express ER and/or PR. These tumors often respond initially to an endocrine therapy that either targets the synthesis of 17β-estradiol (aromatase inhibitors such as Letrozole or Anastrozole) or inhibits ER activity (selective estrogen receptor modulators, SERMs, like Tamoxifen or selective estrogen receptor downregulators, SERDs, like Fulvestrant) [41]. The chapter by Clarke [42] describes a stress

response to these endocrine therapies that is centered on activation of the UPR and its regulation of autophagy and apoptosis. There are multiple ways the UPR can be activated by these drugs including through altered glucose or glutamine metabolism and/or reduced ATP production, each of which may lead to the release of GRP78 and UPR activation. Other sensors of altered energy production including AMPK can also affect the activation of UPR and its control of autophagy and apoptosis. Key features of this integrated stress response may be affected by genes commonly mutated in ER+ breast cancers such as PI3K and AKT. However, unlike HER2 as a driver of HER2+ breast cancer biology, these mutations alone may not be particularly powerful drivers of endocrine responsiveness in many ER+ breast tumors because of complex feedback control signaling that may dampen their signaling. Thus, this chapter describes components of an integrated network that can explain the role of known gene mutations but that does not require these to explain the role of the UPR as an integrator of endocrine responsiveness, and how UPR signaling may be affected by unique features of the ER+ cellular context.

While canonical signaling within the UPR has been well documented, as noted above cellular context can alter how the signals flow through the entire UPR network. Adding to the complexity of UPR-related signaling, several of its key components are regulated by activities that fall outside canonical representations. The insightful chapter by Cook [43] addresses these issues from the perspective of GRP78, the common regulator of each of the three canonical arms of the UPR. Here, the role of plasma membrane bound GRP78, a feature of many cancers [44], is explored in detail. Similarly, GRP78 location on the mitochondria and secreted GRP78 are discussed in the context of their respective contributions to cancer cell biology. These observations have clear implications for how protein subcellular localization may affect the ability to interpret data correctly. Our understanding of the role of immunity in response to cancer and its treatment has begun to advance rapidly in recent years. What is now evident in cancer immunology research is the relative importance of how the UPR may affect multiple aspects of innate and adaptive immunity with clear implications for tumor biology [45]. Hence, the final sections of this chapter begin to address an important feature of the UPR that is less fully covered in the preceding chapters, that is the role of the UPR in immunity.

Several of the earlier chapters in this book have emphasized the critical relationship between the UPR and autophagy. While most chapters have addressed different aspects of the role of the UPR in cancer biology, the inappropriate accumulation of proteins is a notable feature of some neurodegenerative diseases [46, 47]. For example, PERK activation is closely associated with the progression of several neurodegenerative diseases, offering a potential target for therapeutic interventions [48]. The chapter by Moussa [49] provides readers with a perspective beyond cancer by addressing the role of the UPR in the biology of neurodegenerative diseases, with a primary focus on the consequences of its regulation of autophagy. The author carefully introduces the role of an inappropriate accumulation of proteins in neurons. Targeting this accumulation could lead to potentially transformative treatments for diseases that, for the moment, remain intractable therapeutically where the intent is to achieve a cure. Subsequent sections describe the process of autoph-

agy in detail. These sections will be particularly useful to readers unfamiliar with the cellular process of autophagy, independent of the neurodegenerative disease setting in which it is presented here.

Clearly, the UPR plays a critical role in maintaining the balance between the energy needed to fold proteins and, particularly in secretory cells, the need to manage a high load of proteins without compromising cell function and survival. For cancers that arise in secretory tissues, such as breast, prostate, pancreas, salivary gland, and some immune cells, the ability to use the UPR in an attempt to manage the stresses applied from therapeutic interventions is already hard-wired as part of their respective biology [4]. Thus, targeting the UPR, perhaps specifically those features that are uniquely regulated in a cell context-specific manner, may offer the opportunity to develop more effective and more personalized treatments for some of the most common and most lethal cancers.

When taken together, the work presented in this book will provide readers with a detailed but accessible introduction to the UPR and its potential as a target for the development of new anticancer strategies. The section below provides some insight into some future research directions in areas where current knowledge may be limited.

Looking Forward

As is evident from each of the chapters in this book, signal flow through the UPR network is complex. Moreover, the extent to which a canonical representation is useful to understand this signaling, and particularly how this is affected by cell context (including stress-specific responses), is not always clear. Crosstalk among the three UPR signaling arms may also be commonplace and cell context specific, both in nature (e.g., which nodes use which connections) and in time (e.g., when are specific interactions or signaling paths used and when not). As a general guide and as a place to start, static canonical models clearly have their place [16]. However, in dynamic signaling networks with complex feedback and feedforward control and crosstalk among signaling pathways, signaling is often difficult (or impossible) to interpret or predict heuristically. For example, the effects of some signals can be non-linear, where small changes in one component produce a much larger change in another [50]. Mathematical models can be very useful in this setting, particularly where it is important to predict the likely consequences of a measured change in the expression or activation state of key genes. Given the known complexity of signaling and the often sparsity of data, there are relatively few such models of the UPR and these tend to be relatively high level [16, 51]. Moreover, the extent to which canonical signaling is modified by cellular context is often unclear, as is the degree and nature of coordination and integration of signaling crosstalk among the three UPR arms. For complex disease states, including those evident in heterogeneous tumor cell microenvironments, there is likely much yet to be discovered about how the UPR is differentially regulated. Predicting the consequences of UPR activation

for the survival and proliferation of cancer cells, and their adaptive responses to systemic treatment interventions, is an area that likely also requires considerable additional research. This line of research may be greatly facilitated by appropriate computational and mathematical modeling [50].

While activation of the UPR can modify rates of transcription and translation, the proteins a cell needs to function normally, particularly in response to different stressors, likely varies significantly with time and with exposure to cell intrinsic and extrinsic stress. How the UPR is directed to ensure that adequate amounts of the most critical proteins are made in a cell context-specific manner, is another area where there are likely gaps in current knowledge. Indeed, it remains to be seen what level of specificity is required and achieved in this regard beyond those events regulated specifically within the UPR network, at least as this network is understood canonically. Some of these decisions may be made outside the canonical UPR (or the cell) yet affect UPR network signaling. For example, the expression of some genes may be controlled by transcription factors that respond to signals from the cell's microenvironment. In cancer cells, intrinsic events may alter signal flow through the UPR network. Signal flow through a locality of the network may be altered when a driver mutation is acquired during neoplastic transformation or tumor progression. Even if the UPR network connections retain features present in current canonical signaling representations, the weight or importance of each connection may then differ. Changes in the use of different individual nodes is often seen in complex signaling networks that can exhibit small world properties where each node (e.g., mRNA, protein, metabolite) can be reached by connecting through only a small number of other nodes [52]. Thus, there may be cell context-specific preferred signaling routes for UPR activation and execution. Here again, mathematical models may be useful in understanding critical nuances in UPR network signal flow. These issues become more important when looking for drug combinations to block prosurvival signaling through the UPR, as would usually be the case in developing new cancer treatment modalities. Several chapters in this book discuss potential drugs that may be useful in developing new intervention strategies for cancer patients. Developing appropriate drug combinations and schedules may require a greater understanding of UPR signaling, including the dynamical features of signal flow (most signaling is inherently directed and dynamic), and features of redundancy and degeneracy that may confer apparently emergent properties on UPR signaling as are often evident in other signaling networks [50].

Timing of UPR induction is clearly critical if a cell is to be proactive rather than only reactive. Since it is evident that the UPR is not merely a stress response network, it is not surprising that some signals may induce the UPR in advance of the build-up of proteins for folding in the EnR. For example, in secretory cells that receive an external signal to initiate the production of proteins for secretion, waiting for the cell to become stressed from an increased protein load before activating homeostatic regulatory functions could put the cell's ability to survive at risk. Prewiring of the UPR is evident in response to heat shock [19] and is generally called an anticipatory UPR response [53]. Thus, the UPR is activated in advance of increased protein translation [54]. The anticipatory prewiring of key UPR stress

response features is a relatively new and exciting area of research [18]. Moreover, the anticipatory aspects of the UPR are likely to have broad implications beyond the cell models in which they were first reported.

While much of the research described in this book tends to treat the UPR in the context of its action within the EnR, it is evident that the UPR can also be activated in mitochondria [1]. As the primary energy source for a cell, preservation of mitochondrial function is critical for cell survival. Hence, mitochondrial UPR (UPRmt) is induced in response to the accumulation of unfolded or damaged proteins within mitochondria. To maintain integrity and functionality of the mitochondrial proteome, UPRmt activates retrograde signaling that coordinates actions within both the mitochondrial and nuclear genomes [1]. Importantly, the UPRmt ensures continued oxidative phosphorylation through signaling that involves accumulation of ATFS-1 [55]. Perhaps reflecting its role in several aspects of the immune response, UPRmt can initiate a protective innate immune response to eliminate pathogens that attack mitochondrial function [56]. A greater research focus on the UPRmt is anticipated in the near future.

The UPR is a highly coordinated network that controls and/or integrates multiple cellular functions that can support cell development and restore key cellular functions to homeostasis during stress. Whether initiated and executed within the EnR or mitochondria of cancer cells, key components of its signaling offer targets for novel therapeutic intervention. Research into the UPR and its role in cancer biology continues to receive increasing attention. Thus, it is hoped that this volume will provide a useful introduction and reference for its readers.

References

1. Jovaisaite V, Auwerx J. The mitochondrial unfolded protein response-synchronizing genomes. Curr Opin Cell Biol. 2015;33:74–81.
2. Arnould T, Michel S, Renard P. Mitochondria retrograde signaling and the UPR mt: where are we in mammals? Int J Mol Sci. 2015;16:18224–51.
3. Hollien J. Evolution of the unfolded protein response. Biochim Biophys Acta. 2013;1833:2458–63.
4. Clarke R, Cook KL, Hu R, Facey CO, Tavassoly I, Schwartz JL, Baumann WT, Tyson JJ, Xuan J, Wang Y, Warri A, Shajahan AN. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. Cancer Res. 2012;72:1321–31.
5. Shim SM, Choi HR, Sung KW, Lee YJ, Kim ST, Kim D, Mun SR, Hwang J, Cha-Molstad H, Ciechanover A, Kim BY, Kwon YT. The endoplasmic reticulum-residing chaperone BiP is short-lived and metabolized through N-terminal arginylation. Sci Signal. 2018;11. pii: eaan0630.
6. Morito D, Hirao K, Oda Y, Hosokawa N, Tokunaga F, Cyr DM, Tanaka K, Iwai K, Nagata K. Gp78 cooperates with RMA1 in endoplasmic reticulum-associated degradation of CFTRDeltaF508. Mol Biol Cell. 2008;19:1328–36.
7. Buchberger A, Bukau B, Sommer T. Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell. 2010;40:238–52.

8. Shiber A, Ravid T. Chaperoning proteins for destruction: diverse roles of Hsp70 chaperones and their co-chaperones in targeting misfolded proteins to the proteasome. Biomol Ther. 2014;4:704–24.
9. Ding WX, Yin XM. Sorting, recognition and activation of the misfolded protein degradation pathways through macroautophagy and the proteasome. Autophagy. 2008;4:141–50.
10. Clarke R, Shajahan AN, Wang Y, Tyson JJ, Riggins R, Weiner LM, Baumann WT, Xuan J, Zhang B, Facey C, Aiyer H, Cook K, Hickman FE, Tavassoly I, Verdugo A, Chen C, Zwart A, Wärri A, Hilakivi-Clarke LA. Endoplasmic reticulum stress, the unfolded protein response, and gene network modeling in antiestrogen resistant breast cancer. Horm Mol Biol Clin Invest. 2011;5:35–44.
11. Back SH, Kaufman RJ. Endoplasmic reticulum stress and type 2 diabetes. Annu Rev Biochem. 2012;81:767–93.
12. Scheuner D, Kaufman RJ. The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. Endocr Rev. 2008;29:317–33.
13. Mohrin M, Widjaja A, Liu Y, Luo H, Chen D. The mitochondrial unfolded protein response is activated upon hematopoietic stem cell exit from quiescence. Aging Cell. 2018;17:e12756.
14. Sigurdsson V, Miharada K. Regulation of unfolded protein response in hematopoietic stem cells. Int J Hematol. 2018;107:627.
15. Scheckel C, Aguzzi A. Prions, prionoids and protein misfolding disorders. Nat Rev Genet. 2018;19:405–18.
16. Tyson JJ, Baumann WT, Chen C, Verdugo A, Tavassoly I, Wang Y, Weiner LM, Clarke R. Dynamic modeling of oestrogen signalling and cell fate in breast cancer cells. Nat Rev Cancer. 2011;11:523–32.
17. Rajapaksa G, Thomas C, Gustafsson JA. Estrogen signaling and unfolded protein response in breast cancer. J Steroid Biochem Mol Biol. 2016;163:45–50.
18. Livezey M, Kim JE, Shapiro DJ. A new role for estrogen receptor alpha in cell proliferation and cancer: activating the anticipatory unfolded protein response. Front Endocrinol. 2018;9:325.
19. Vihervaara A, Duarte FM, Lis JT. Molecular mechanisms driving transcriptional stress responses. Nat Rev Genet. 2018;19:385–97.
20. Kondratyev M, Avezov E, Shenkman M, Groisman B, Lederkremer GZ. PERK-dependent compartmentalization of ERAD and unfolded protein response machineries during ER stress. Exp Cell Res. 2007;313:3395–407.
21. Tsai YC, Weissman AM. The unfolded protein response, degradation from endoplasmic reticulum and cancer. Genes Cancer. 2010;1:764–78.
22. Kleiger G, Mayor T. Perilous journey: a tour of the ubiquitin-proteasome system. Trends Cell Biol. 2014;24:352–9.
23. Kaushik S, Cuervo AM. The coming of age of chaperone-mediated autophagy. Nat Rev Mol Cell Biol. 2018;19:365–81.
24. Fujita E, Kouroku Y, Isoai A, Kumagai H, Misutani A, Matsuda C, Hayashi YK, Momoi T. Two endoplasmic reticulum-associated degradation (ERAD) systems for the novel variant of the mutant dysferlin: ubiquitin/proteasome ERAD(I) and autophagy/lysosome ERAD(II). Hum Mol Genet. 2007;16:618–29.
25. Obacz J, Avril T, Le Reste PJ, Urra H, Quillien V, Hetz C, Chevet E. Endoplasmic reticulum proteostasis in glioblastoma-from molecular mechanisms to therapeutic perspectives. Sci Signal. 2017;10. pii: eaal2323.
26. Dominicus CS, Patel V, Chambers JE, Malzer E, Marciniak SJ. Endoplasmic reticulum stress signaling during development. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
27. Arensdorf AM, Diedrichs D, Rutkowski DT. Regulation of the transcriptome by ER stress: non-canonical mechanisms and physiological consequences. Front Genet. 2013;4:256.
28. Morreal J, Hong F, Li Z. Regulation of the unfolded protein response and its roles in tumorigenesis and cancer therapy. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.

29. Bu Y, Diehl JA. PERK integrates oncogenic signaling and cell survival during cancer development. J Cell Physiol. 2016;231:2088–96.
30. Xie H, Tang CH, Song JH, Mancuso A, Del Valle JR, Cao J, Xiang Y, Dang CV, Lan R, Sanchez DJ, Keith B, Hu CC, Simon MC. IRE1alpha RNase-dependent lipid homeostasis promotes survival in Myc-transformed cancers. J Clin Invest. 2018;128:1300–16.
31. Shajahan-Haq AN, Cook KL, Schwartz-Roberts JL, Eltayeb AE, Demas DM, Warri AM, Facey CO, Hilakivi-Clarke LA, Clarke R. MYC regulates the unfolded protein response and glucose and glutamine uptake in endocrine resistant breast cancer. Mol Cancer. 2014;13:239.
32. Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia. 2015;3:83–92.
33. Fels DR, Koumenis C. The PERK/eIF2alpha/ATF4 module of the UPR in hypoxia resistance and tumor growth. Cancer Biol Ther. 2006;5:723–8.
34. Singleton DC, Harris AL. ATF4, hypoxia and treatment resistance in cancer. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
35. Sengupta S, Jordan VC, Clarke R. Role of protein translation in the unfolded protein response. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
36. Aarti I, Rajesh K, Ramaiah KV. Phosphorylation of eIF2 alpha in Sf9 cells: a stress, survival and suicidal signal. Apoptosis. 2010;15:679–92.
37. Hu R, Clarke R. Roles of spliced and unspliced XBP1 in breast cancer. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
38. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13:674–90.
39. Jamdade VS, Sethi N, Mundhe NA, Kumar P, Lahkar M, Sinha N. Therapeutic targets of triple-negative breast cancer: a review. Br J Pharmacol. 2015;172:4228–37.
40. Zhao N, Peng F, Chen X. The unfolded protein response in triple-negative breast cancer. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
41. Clarke R, Tyson JJ, Dixon JM. Endocrine resistance in breast cancer – an overview and update. Mol Cell Endocrinol. 2015;418:220–34.
42. Clarke R. The unfolded protein response as an integrator of response to endocrine therapy in estrogen receptor positive breast cancer. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
43. Cook KL. Outside the endoplasmic reticulum: non-canonical GRP78 signaling. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
44. Schwarze S, Rangnekar VM. Targeting plasma membrane GRP78 for cancer growth inhibition. Cancer Biol Ther. 2010;9:153–5.
45. Pommier A, Anaparthy N, Memos N, Kelley ZL, Gouronnec A, Yan R, Auffray C, Albrengues J, Egeblad M, Iacobuzio-Donahue CA, Lyons SK, Fearon DT. Unresolved endoplasmic reticulum stress engenders immune-resistant, latent pancreatic cancer metastases. Science. 2018;360. pii: eaao4908.
46. Scheper W, Hoozemans JJ. The unfolded protein response in neurodegenerative diseases: a neuropathological perspective. Acta Neuropathol. 2015;130:315–31.
47. Scheper W, Hoozemans JJ. A new PERKspective on neurodegeneration. Sci Transl Med. 2013;5:206fs37.
48. Hughes D, Mallucci GR. The unfolded protein response in neurodegenerative disorders - therapeutic modulation of the PERK pathway. FEBS J. 2018.
49. Moussa C. Autophagy and the unfolded protein response in neurodegenerative diseases. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
50. Clarke, R., Tyson, J. J., Tan, M., Baumann, W. T., Xuan, J., and Wang, Y. Systems biology: perspectives on multiscale modeling in research on endocrine-related cancers. Endocr Relat Cancer. 2018; in revision.
51. Parmar JH, Cook KL, Shajahan-Haq AN, Clarke PA, Tavassoly I, Clarke R, Tyson JJ, Baumann WT. Modelling the effect of GRP78 on anti-oestrogen sensitivity and resistance in breast cancer. Interface Focus. 2013;3:20130012.

52. Ma'ayan A. Insights into the organization of biochemical regulatory networks using graph theory analyses. J Biol Chem. 2009;284:5451–5.
53. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334:1081–6.
54. Shapiro DJ, Livezey M, Yu L, Zheng X, Andruska N. Anticipatory UPR activation: a protective pathway and target in Cancer. Trends Endocrinol Metab. 2016;27:731–41.
55. Nargund AM, Fiorese CJ, Pellegrino MW, Deng P, Haynes CM. Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPR(mt). Mol Cell. 2015;58:123–33.
56. Pellegrino MW, Nargund AM, Kirienko NV, Gillis R, Fiorese CJ, Haynes CM. Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. Nature. 2014;516:414–7.

Chapter 2
Endoplasmic Reticulum Stress Signalling During Development

Caia S. Dominicus, Vruti Patel, Joseph E. Chambers, Elke Malzer, and Stefan J. Marciniak

Abstract The unfolded protein response (UPR) is a complex homeostatic programme that balances the load of secretory protein synthesis with the folding capacity of the endoplasmic reticulum (ER). Although originally believed to function predominantly as a stress response pathway, growing evidence supports a role for the UPR in the regulation of development. The study of human diseases alongside work with transgenic mouse models has implicated the UPR in a wide range of developmental processes. This chapter examines the three distinct branches of the UPR and their importance during development.

Keywords ER stress · Development · PERK · IRE1 · ATF6

C. S. Dominicus
Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

The Francis Crick Institute, London, UK
e-mail: caia.dominicus@crick.ac.uk

V. Patel
Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

University College London, London, UK

J. E. Chambers · S. J. Marciniak (✉)
Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK
e-mail: sjm20@cam.ac.uk

E. Malzer
Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK

MRC Harwell Institute Oxfordshire, England, UK

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_2

Introduction

The Unfolded Protein Response

In eukaryotic cells, proteins destined for secretion or cell-surface exposure undergo post-translational modifications, such as oxidative folding and N-glycosylation, to ensure their stability in extracellular conditions [1]. This maturation process is incompatible with the cytosolic environment and so eukaryotes evolved an extracellular-like compartment called the endoplasmic reticulum (ER) in which such folding can occur [2]. Newly synthesized polypeptides are translocated into the lumen of the ER via a proteinaceous pore, the Sec61 complex, and subsequently undergo chaperone-assisted folding and post-translational modifications. The ER maintains a stringent quality control system that ensures only properly folded proteins progress down the secretory pathway, while unfolded and misfolded proteins are retained and ultimately returned to the cytoplasm for ER-associated degradation (ERAD) [3] (Fig. 2.1). Aberrant protein folding can lead to the formation of insoluble aggregates through non-native protein–protein interactions, and this has been linked to many clinical disorders including cancer [4–8]. In light of the deleterious consequences of protein misfolding, it is crucial that cells adapt to an imbalance between the ER's folding capacity and the appearance of unfolded client proteins (so-called ER stress). The response elicited by the accumulation of unfolded or misfolded proteins is known as the unfolded protein response (UPR; Fig. 2.1).

The UPR constitutes a three-pronged signalling pathway that (1) attenuates the rate of mRNA translation thus lowering the levels of new ER client proteins [9]; (2) induces the transcription of target genes that increase the folding capacity of the ER or enhance degradation of aberrant proteins [10]; and (3) triggers cell death if the levels of ER stress prove to be insurmountable [11, 12]. The entire response is mediated by three ER stress transducers: inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6), and protein kinase RNA (PKR)-like ER kinase (PERK), which will be considered in turn.

IRE-1

IRE1 is found in all eukaryotes and, accordingly, regulates the most evolutionary conserved arm of the UPR. Mammals possess two IRE1 paralogues, IRE1α and IRE1β [13–15], of which IRE1α is expressed ubiquitously, while IRE1β is restricted to the intestinal and pulmonary epithelia [14]. In most tissues, IRE1 signalling is mediated primarily by the IRE1α isoform, while IRE1β expression is more restricted, being required for intestinal and airway epithelial mucin production [16, 17]. One model suggests that IRE1 activation is regulated by the binding of the HSP70-class chaperone Binding immunoglobulin Protein (BiP) to its ER luminal domain [17]. During ER stress, BiP binds to misfolded proteins, releasing IRE1 for

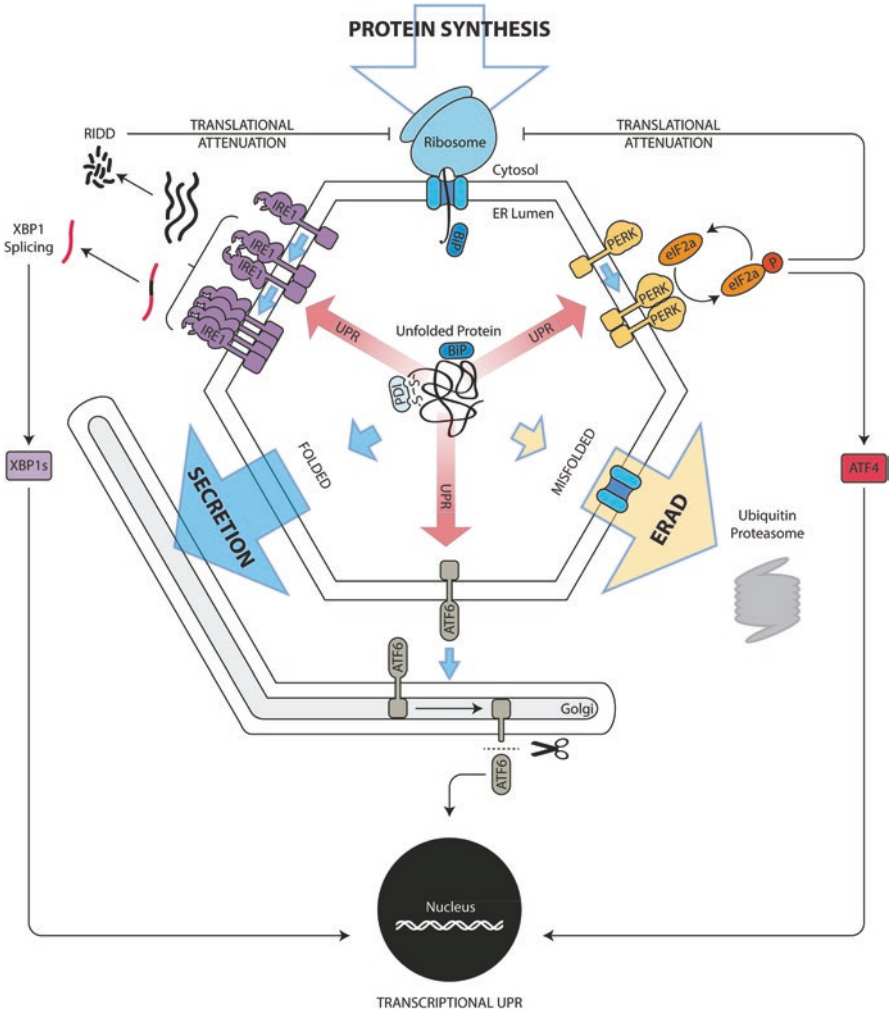

Fig. 2.1 Proteostasis in the endoplasmic reticulum (ER). Polypeptide chains enter the ER cotranslationally and associate with ER chaperones including BiP and PDI. Correctly folded proteins exit the ER while misfiled proteins are retained and may eventually be targeted for ER-associated degradation (ERAD). Accumulation of unfolded proteins triggers the unfolded protein response (UPR) via IRE1, ATF6, and PERK. During ER stress, IRE1 initiates unconventional splicing of XBP1 mRNA, while ATF6 traffics to the Golgi where proteolysis liberates its transcription factor domain. Both XBP1 and cleaved ATF6 up-regulate components of the ER folding and degradation machineries. PERK phosphorylates eIF2α to reduce global protein synthesis while inducing the transcription factor ATF4. ATF4 promotes oxidative protein folding, amino acid synthesis, and eventual recovery of normal rates of translation. IRE1 can also reduce translation by degrading ER-localized mRNAs via regulated IRE1-dependent decay (RIDD). Adapted from [1]

oligomerization and activation. However, structural studies have suggested that the luminal domain of yeast IRE1 may adopt a conformation similar to the peptide-binding cleft of major histocompatibility complex (MHC) [18]. This raises the intriguing possibility that IRE1 might bind directly to misfolded protein polypeptides. These two competing models are not mutually exclusive.

Upon activation, IRE1 oligomerizes and trans-autophosphorylates its cytosolic kinase domain [19]. The resulting conformational change leads to activation of the endoribonuclease domain of IRE1 resulting in specific cleavage of a single mRNA substrate, X-box binding protein-1 (XBP1) [20]. IRE1 catalyses the excision of an inhibitory intron from this mRNA, which is then religated by RtcB [20–22]. The resulting frame shift changes the sequence of the C-terminal portion of XBP1 to generate a functionally active transcription factor (XBP1s). Transcriptional targets of XBP1s mediate increased ER folding capacity, membrane biogenesis, and autophagy [23, 24].

Recently, the endoribonuclease activity of IRE1 has been implicated in an additional process termed 'regulated IRE1 dependent decay' (RIDD) [25]. In response to prolonged stress, IRE1 cleaves a subset of mRNAs encoding predominantly membrane and secreted proteins, thus alleviating ER client load. Substrate specificity for this more promiscuous cleavage mechanism appears to be directed by both ER localization and some degree of transcript sequence specificity [26, 27]. Surprisingly, *in vitro* experiments have suggested that XBP1 mRNA splicing and RIDD may occur independently of one another. Drug-mediated activation of an engineered IRE1 has been shown to activate XBP1-directed nuclease activity without triggering RIDD [25, 28]. It remains to be determined how IRE1 selects between these two activities. As both dimers and higher order IRE1 oligomers have been detected during ER stress [28, 29], it is plausible that the state of IRE1 oligomerization may play a role in regulating its endoribonuclease substrate selection.

PERK

PERK regulates the second, and evolutionarily younger, arm of the mammalian UPR. Its luminal domain shares 20% identity with IRE1, while its cytoplasmic kinase domain shares 40% identity to RNA-dependent protein kinase (PKR), a known eukaryotic initiation factor 2α (eIF2α) kinase [9]. This led to the discovery that while PERK is activated in a manner similar to that of IRE1, following its activation PERK phosphorylates eIF2α on serine 51 [9]. Interestingly, while dispensable for its catalytic activity, phosphorylation of PERK's insert loop domain is required for eIF2α recruitment [30]. During the initiation of mRNA translation, the eIF2 complex recruits aminoacylated initiation methionyl-transfer RNA (met-$tRNA_i^{met}$) to the ribosome in a GTP-dependent manner [31]. This process involves hydrolysis of GTP, and subsequent initiation events depend on the exchange of GDP for GTP by the guanine nucleotide exchange factor eIF2B. Phosphorylation of eIF2α inhibits eIF2B, preventing the GDP to GTP exchange from occurring, thus

causing the initiation complex to be held in an inactive conformation and global translation to be attenuated.

In addition to a downturn in global translation, phosphorylated eIF2α induces the preferential translation of a subset of transcripts by overcoming the inhibitory effects of small upstream open reading frames [32, 33]. A key effector that is up-regulated in this manner is the activating transcription factor 4 (ATF4) [32]. This contributes to the cell's adaptation to stress, since ATF4 transactivates numerous genes encoding chaperones, amino acid transporters, and antioxidants [10]. ATF4 also induces expression of another transcription factor, C/EBP homologous protein (CHOP), which is required for the up-regulation of various genes including the regulatory subunit of the protein phosphatase 1 (PP1), growth arrest and damage gene 34 (GADD34, also known as PPP1R15A) [12, 34]. GADD34 directs PP1 to dephosphorylate eIF2α, allowing for the resumption of normal rates of translation [35–37]. The ATF4-CHOP-GADD34 axis plays a role in the resolution of ER stress because it enables the recovery of global translation, facilitating the protein synthesis necessary for translation of mRNAs up-regulated during the UPR.

ATF6

ATF6α is an ER type II transmembrane protein that triggers a third arm of the mammalian UPR. ATF6β is a distant homologue of ATF6α, and both are widely expressed in all tissues. Under ER stress conditions, BiP dissociates from the luminal domain of ATF6 to reveal a Golgi localization signal that allows it to exit the ER and traffic to the Golgi apparatus [38]. Furthermore, recent studies have demonstrated that disulphide bonds within ATF6 are reduced during ER stress, and that the degree of reduction correlates with activation [39]. While reduction of disulphide bonds does not appear to be directly responsible for activation, fractionation studies have demonstrated that only reduced monomeric ATF6 reaches the Golgi where it is cleaved by Site-1 (S1P) and Site-2 proteases (S2P) to produce a soluble cytosolic fragment, ATF6(N) [38–40]. This truncated form migrates to the nucleus where it transactivates numerous ER-stress responsive genes, including BiP, CHOP, and XBP1 [41, 42].

UPR in Physiological Development

Although frequently considered to function exclusively as a stress response pathway, much evidence supports a role for the UPR during physiological development. Various human diseases and mouse studies have implicated UPR mediators in developmental pathways and many appear to be essential for embryonic development, as evidenced by embryonic knockout lethality (Table 2.1). Moreover, perturbation of these pathways disrupts tissue development in animal models [63].

Table 2.1 Genetic models linking the unfolded protein response with development

UPR component	Genetic model	Phenotype	Reference(s)
IRE1α	*Ire1α*$^{-/-}$	Lethal between embryonic days (E) 12.5 and 13 Marked vascularization defects in the placenta	[43] [44]
	Ire1α/Rag2$^{-/-}$	Defects in early and late stages of plasma-cell differentiation	[43]
XBP1	*Xbp1*$^{-/-}$	Embryonic lethal at E14.5 with severe liver hypoplasia and reduced haematopoiesis causing severe anaemia	[45]
	Liver-specific expression of *Xbp1* (*Xbp1*$^{-/-}$;*Liv*^{Xbp1})	Impaired exocrine pancreatic function	[46]
	Xbp1/Rag2$^{-/-}$	Defective late-stage plasma-cell differentiation Defective dendritic cell differentiation	[47] [48]
PERK	*Perk*$^{-/-}$	Hyperglycaemia in 4-week old mice due to degeneration of insulin secreting ß-cells Severe osteopenia	[49] [50]
eIF2α	Homozygous nonphosphorylatable knock-in (*eIF2α*$^{S51A/S51A}$)	Severe hypoglycaemia during the first post-natal day and die within 18 h	[51]
	Heterozygous nonphosphorylatable knock-in (*eIF2α*$^{S51A/+}$)	Increased obesity and adipocyte number, glucose intolerance, and impaired insulin secretion when placed on a high-fat diet. β-cell failure	[52] [53]
ATF4	*Atf4*$^{-/-}$	Severe osteopenia Severe cartilage defects	[54] [55]
CHOP	*Chop*$^{-/-}$	Decreased bone formation Note: it appears that CHOP has a complex role in osteoblast function, both inhibiting differentiation in some settings, but able to promote BMP signalling in a cell-type specific manner	[56] [57] [58]
		Increased body fat mass relative to control when placed on high fat diet	[53]
ATF6	*Atf6α/B*$^{-/-}$	Embryonic lethal	[59]
	Atf6α$^{-/-}$	Older mice develop marked degeneration of photoreceptors	[60]
OASIS	*Oasis*$^{-/-}$	Severe osteopenia	[61]
	Cerebralcortex-specific*Oasis*$^{-/-}$	Cerebral cortices contain fewer astrocytes and more neural precursor cells	[62]

In this chapter, we will examine each branch of the UPR and their roles in development.

IRE1-XBP1 Axis in Development

Embryonic Liver and Placenta

Knockout mouse models have demonstrated a key role for IRE1α in embryonic development [43, 64]. *Ire1α*-deficient mice die between embryonic days (E) 12.5 and 13, at which point they show marked growth retardation, liver hypoplasia, and anaemia [43]. This outcome likely reflects impaired processing of *Xbp1* mRNA since whole-animal *Xbp1* deletion is also embryonic lethal at E14.5 with severe liver hypoplasia and reduced haematopoiesis [45]. This embryonic lethality can be rescued if XBP1 is re-expressed specifically within the liver [46]. The effects on haematopoiesis appear to reflect a requirement of the developing liver for XBP1 in order to provide an appropriate environment for the establishment of red cell precursors, rather than a direct effect on these precursors themselves. For example, in normal mice, high levels of *Xbp1* mRNA are detected in the liver bud at E10.5, before significant population of the liver by haematopoietic stem cells [45]. Moreover, XBP1-deficient haematopoietic progenitor cells derived from fetal aorto-gonad-mesonephros, yolk sac, or liver all show normal proliferation and differentiation *in vitro* [45]. The mechanism of this effect remains to be fully elucidated, although microarray experiments have suggested a number of hepatocyte-specific target genes of XBP1 including *α-fetoprotein*, a putative regulator of hepatocyte growth, and three acute phase protein family members, *α_1-antitrypsin*, *transthyretin,* and *apolipoprotein A1,* which were each found at significantly reduced levels in XBP1-deficient cells [45].

The importance of IRE1α during development is not restricted to the liver. Bioluminescence imaging of an XBP1-luciferase reporter identified intense IRE1 activity at embryonic day 13, primarily in the placenta [44]. Accordingly, high levels of *Ire1*α and spliced *Xbp1* (*Xbp1s*) mRNA were detected in the placenta from E8.5 to E15.5 [44]. At E13.5, the placenta is formed of three readily discernible layers: the labyrinth, spongiotrophoblast, and decidua; but in *Ire1*$\alpha^{-/-}$ mice, only the labyrinth is abnormal, showing marked vascularization defects [44]. This layer of the placenta, which is the site of oxygen and nutrient exchange between mother and fetus, also showed decreased expression of vascular endothelial growth factor A (VEGF-A), a key factor required for placental vascularization [44, 65]. Remarkably, XBP1-deficient mice showed no such alteration in VEGF-A expression suggesting the existence of other IRE1 targets [44]. The importance of placental IRE1α is striking since deletion of *Ire1α* in all tissues apart from trophoblasts of the labyrinth generates mice with normal VEGF-A and proper placental development [44]. Curiously, not only do these animals survive gestation, but they also show rescue of

the liver hypoplasia seen previously in *Ire1α*$^{-/-}$ animals. It remains unclear whether expression of IRE1α within the placenta rescues liver hypoplasia by compensating for nutrient deprivation of the whole fetus, or whether the IRE1α-deficient liver can compensate for deficient IRE1-XBP1 signalling if provided with sufficient nutrition.

Nevertheless, the requirement for IRE1α signalling during embryogenesis suggests a critical role for the UPR in placental angiogenesis, while the XBP1-independent effects of IRE1α on VEGF-A expression suggest the existence of further important IRE1 targets although their identities remain obscure. Transcriptional profiling has identified carcinoembryonic antigen (CEA) family genes *Ceacan* and *Psg* to be dysregulated in *Ire1α*$^{-/-}$ placentas [66]. While these are plausibly involved in regulating VEGF-A expression and/or immunomodulation within the placenta [66, 67], they appear to be XBP1 dependent and so other important targets probably exist.

Pancreas and Stomach

Although liver-specific expression of *Xbp1* (*Xbp1*$^{-/-}$;*Liv*^{Xbp1}) rescued the embryonic lethality of *Xbp1*$^{-/-}$ mice, these animals went on to die in the perinatal period with signs of growth retardation, nutritional failure, and hypoglycaemia [46]. The mutant pups showed distended loops of bowel filled with undigested milk suggesting impaired exocrine pancreatic function [46] consistent with previous observations that XBP1 and IRE1 are highly expressed in the pancreas [13, 68]. Indeed, the pancreata of *Xbp1*$^{-/-}$;*Liv*^{Xbp1} mice were much smaller than those of wild-type controls, with sparsely distributed acini in a loose mesenchymal background [46]. As one might expect from the known requirement of XBP1 for ER expansion, the acinar cells of mutant animals had a poorly developed ER and a marked reduction in the number and size of zymogen secretory granules [46]. In keeping with this, the mutant pancreata contained little trypsinogen and the expression of amylase and elastase was reduced [46]. There is an ongoing requirement for IRE1-XBP1 signalling for pancreatic health since conditional disruption of XBP1 in adult pancreatic acinar cells results in an identical phenotype [69]. Interestingly, loss of XBP1 did not affect embryonic pancreatic islet cell development, suggesting that XBP1 is required primarily by the exocrine pancreas [46]. The expression of a number of important ER proteins is reduced in the *Xbp1*$^{-/-}$;*Liv*^{Xbp1} pancreas, including Sec61α, EDEM, PDI, and the exocrine pancreas-specific isoform PDIp [46, 70, 71]. Sec61α plays a role in protein translocation across the ER membrane [72], while EDEM is involved in the degradation of misfolded ER proteins [73]. The PDI proteins both catalyse disulphide bond formation and the isomerization of newly synthesized proteins [74].

XBP1 has also been shown to regulate directly the expression of *Mist1*, a key gene in the development of the exocrine pancreas and the regulation of secretory vesicle maturation [75, 76]. XBP1 was found to bind the *Mist1* promoter in various

cell lines, and XBP1 over-expression induced expression of *Mist1* [77]. While these studies were not performed in acinar cells, it is plausible that XBP1 regulates *Mist1* expression in the pancreas and that a defect in this regulation accounts for some of the exocrine pancreatic defects seen in $Xbp1^{-/-};Liv^{Xbp1}$ mice.

Through effects on *Mist1,* XBP1 also regulates differentiation of gastric zymogenic cells from mucous neck-cells [78]. Conditional deletion of *Xbp1* in adult stomachs using a tamoxifen-sensitive cre-lox system led to zymogenic cell-specific deficits of the ER and granulogenesis that closely resembled MIST1 loss of function in the exocrine pancreas [78–80]. Again, XBP1 was confirmed to bind directly to the promoter of *Mist1* [78]. Interestingly, loss of XBP1 appeared to impair the loss of progenitor neck-cell genes by zymogenic cells [78]. *Xbp1* null zymogenic cells remained stuck in a state of transition, unable to differentiate terminally. This increase in the expression of neck-cell markers is reminiscent of the transitional morphology of zymogenic lineages seen in spasmolytic polypeptide-expressing metaplasia (SPEM) [79]. In this gastric precancerous lesion, the loss of MIST1 expression is one of the first markers of defective neck-cell to zymogenic cell differentiation [81].

B Cells and Plasma Cells

The importance of the UPR in plasma cell differentiation was first highlighted in studies focused on B cell lymphopoiesis and observations that XBP1 is up-regulated in myeloma cell lines [82]. XBP1s and ATF6c are both generated during B cell differentiation, while the PERK axis appears to remain quiescent [83]. Loss of XBP1 is embryonically lethal, but use of a RAG2 complementation system enabled the generation of mice with selective loss of XBP1 from lymphocytes [84]. *Xbp1-Rag-*$2^{-/-}$ lymphoid chimeric mice developed normally, generated normal numbers of B and T cells, secreted cytokines and formed normal germinal centres [84]. However, XBP1-deficient animals had significantly lower baseline levels of all serum immunoglobulin isotypes. When challenged with T cell-independent or dependent antigens, those mice failed to mount an effective antibody response [84]. Similarly, animals deficient in XBP1 in lymphoid cells were markedly impaired in their ability to control infection with polyoma virus, which is usually cleared through B cell-mediated immunity [84]. Stimulation of B cells with lipopolysaccharide (LPS) or interleukin-4 has been shown to induce expression of *Xbp1* mRNA and its IRE1-dependent splicing [85]. Moreover, the expression of UPR targets such as BiP and GRP94 was increased in LPS-stimulated B cells prior to the translation of immunoglobin chains [83, 85]. Indeed, B cell receptor signalling is sufficient to induce a short-lived UPR [86].

The precise role of XBP1 activation in B cell development has been subject to some debate. Early studies using chimeric *Xbp1-Rag-*$2^{-/-}$ mice found that expression of CD138 (syndecan-1), a marker of plasma cell differentiation, could not be detected on the surface of XBP1-deficient B cells after stimulation [84]. This led to

the conclusion that XBP1 is required, not only for the proper secretory function of plasma cells, but also for their differentiation. More recent studies using a B cell-specific *Xbp1* knockout mutant mouse confirmed that XBP1 is required for secretion of immunoglobins. However, those mice exhibited only minor effects on plasma cell differentiation that were restricted to later stages of plasma cell development [47, 87, 88]. This incongruity with previous findings may be accounted for by the fact that XBP1 has also been shown to play a role in the development and function of dendritic cells [48]. It is therefore conceivable that XBP1 function in a non-B cell population has an impact on the numbers of $CD138^+$ cells in *Xbp1-Rag-2*$^{-/-}$ mice [87].

It is now clear that XBP1 works within a larger transcriptional circuit that governs B cell proliferation and plasma cell differentiation [89]. BCL-6 and Pax5 are factors that promote proliferation of B cells, while blocking differentiation to plasma cells (Fig. 2.2). Pax5 blocks expression of XBP1 [90], while BCL-6 blocks BLIMP1, a master regulator of plasma cell differentiation [91]. BLIMP1, in turn, suppresses expression of both BCL-6 and Pax5, thus blocking further proliferation and de-repressing of XBP1 expression [92, 93]. Independent of BLIMP1, IRF4 also promotes plasma cell differentiation and permits XBP1 expression [94]. In *Xbp1*$^{-/-}$ cells, both BLIMP1 and IRF4 levels have been reported to increase, suggesting that XBP1 or its targets feedback to these factors (Fig. 2.2) [47, 87]. The loss of XBP1 from B cells appears to impair B cell receptor signalling leading to aberrant IRF4 and BLIMP1 induction [47]. Although these XBP1-deficient B cells form antibody-secreting plasmablasts following immunization, they fail to undergo CXCL12-induced chemotaxis to the bone marrow, and are unable to sustain antibody secretion [47]. It is worth noting that one report has described only modest effects on antibody secretion by *Xbp1*$^{-/-}$ cells and normal expression of BLIMP1 and IRF4 [88]. The reason for this disparity is not yet clear and so additional studies are needed.

Interestingly, IRE1 deficiency also leads to B lymphopoietic defects, but at an earlier developmental stage than observed for XBP1-deficient cells. *Ire1α*$^{-/-}$ haematopoietic stem cells can commit to the lymphoid lineage, but do not progress beyond the pro-B cell stage of development [43]. These cells are severely defective in VDJ recombination of immunoglobulin genes and so fail to express B cell receptors [43]. This defect has been attributed to a role for IRE1 in regulating the expression of the recombination-activating genes *rag1*, *rag2*, and the gene encoding terminal deoxynucleotidyl transferase (TdT) in pro-B cells, rather than an effect on XBP1 *per se*. The kinase or endoribonuclease activities of IRE1 are not necessary for this effect, which is difficult to reconcile with current models of IRE1 function [43, 84]. Recent advances in techniques with which to study the IRE1-XBP1 axis warrant renewed investigation of this hypothesis.

In keeping with their importance in normal plasma cell development, expression levels of IRE1 and XBP1s are markedly increased in multiple myeloma, and XBP1s has been identified as an independent prognostic marker and predictor of thalidomide response in this disease [95]. Moreover, inhibition of the IRE1 endoribonuclease domain by the small molecule MKC-3946 was found to trigger a modest growth

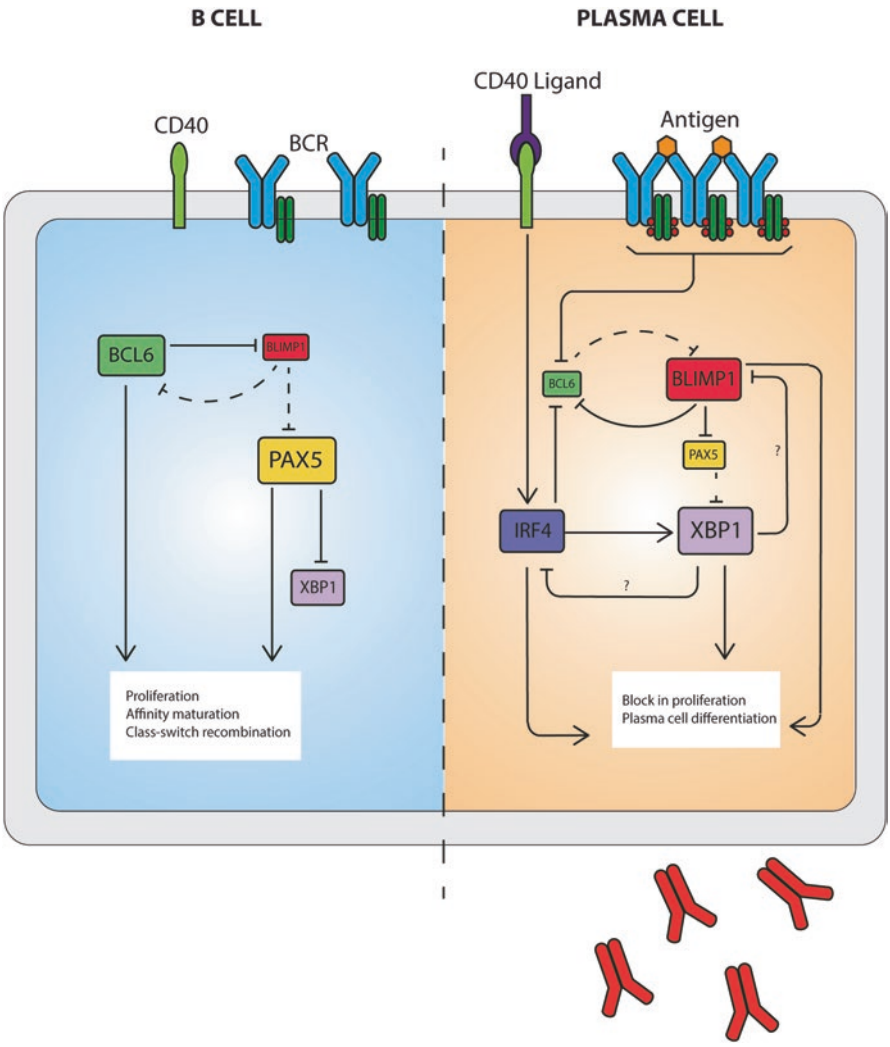

Fig. 2.2 B cell differentiation. Transcription factors PAX5, BCL-6, BLIMP1, IRF4, and XBP1 form a circuit regulating B cell proliferation and plasma cell differentiation. BCL-6 blocks expression of BLIMP1, the master regulator of plasma cell differentiation, and promotes B cell proliferation. Signalling through the B cell receptor (BCR) leads to degradation of BCL-6, de-repressing of BLIMP1. BLIMP1 suppresses expression of PAX5 to de-repress XBP1, which promotes expansion of the ER and enables increased secretion of immunoglobulin by plasma cells. Activation of the CD40 receptor leads to NF-κβ-mediated induction of IRF4, which suppresses BCL-6 expression, promotes plasma cell differentiation, and permits XBP1 expression. XBP1 might acts as a negative feedback regulator of both BLIMP1 and IRF4

inhibition in several myeloma cell lines, without affecting normal mononuclear cells [96].

Dendritic Cells

The IRE1α-XBP1 pathway is also important for the development of dendritic cells, another key component of the immune system [48]. These are critical in orchestrating the innate and adaptive immune responses by presenting antigens to T and B cells in the secondary lymphoid tissues. Their survival is tightly controlled by various stimuli, including pathogen-mediated Toll-like receptor (TLR) stimulation. *Xbp1-Rag-2*$^{-/-}$ chimeric mice show a marked reduction in the numbers of conventional dendritic cells and, particularly, plasmacytoid dendritic cells, a subset characterized by an expanded ER and high interferon type 1 (IFN-α) secretion [48, 97]. Both conventional and plasmacytoid dendritic cells from XBP1-deficient mice show reduced survival upon TLR engagement, while the plasmacytoid dendritic cells fail to expand their ER and are impaired in IFN-α production [48]. All freshly isolated dendritic cell subsets show elevated *XBP1* splicing relative to unactivated T and B lymphocytes, with especially high levels of total *XBP1* mRNA seen in plasmacytoid dendritic cells [48]. Even in the absence of additional ER stress, IRE1α activation and *XBP1* splicing can be detected [98]. Subsequent TLR stimulation does not significantly affect *Xbp1* mRNA expression or splicing, suggesting that XBP1 governs an earlier stage of dendritic cell development distinct from the TLR-induced nuclear factor-κB (NF-κB)-dependent pathway [48]. Dendritic cells differentiate from Fms-like tyrosine kinase 3 (Flt3)-expressing cells, but the numbers of Flt3^{+} cells are not reduced in *Xbp1*$^{-/-}$ haematopoietic progenitors [48, 99]. Forced expression of XBP1s in Flt3^{+} but not Flt3^{-} progenitors rescues both dendritic cell subsets [48]. Indeed, high levels of XBP1s expression can render *Xbp1*$^{-/-}$ cells more efficient at dendritic cell differentiation relative to wild-type controls. This observation suggests that XBP1-mediated effects on differentiation must occur after the Flt3^{+} progenitor stage. Loss of *Xbp1*$^{-/-}$ has complex effects on dendritic cell biology, not only through loss of target gene expression, but also through loss of feedback inhibition of IRE1α. In dendritic cells lacking functional XBP1, high levels of RIDD altered their phenotype by enhancing the degradation of mRNAs encoding components of the MHC class I machinery and CD18 integrins [98].

Osteogenesis

XBP1 may also play a role in bone development. Mouse embryonic fibroblasts treated with bone morphogenic protein (BMP)-2 show marked increases in expression and splicing of XBP1. In the absence of IRE1, treatment with BMP2 failed to cause the expected increase of alkaline phosphatase activity or expression of the

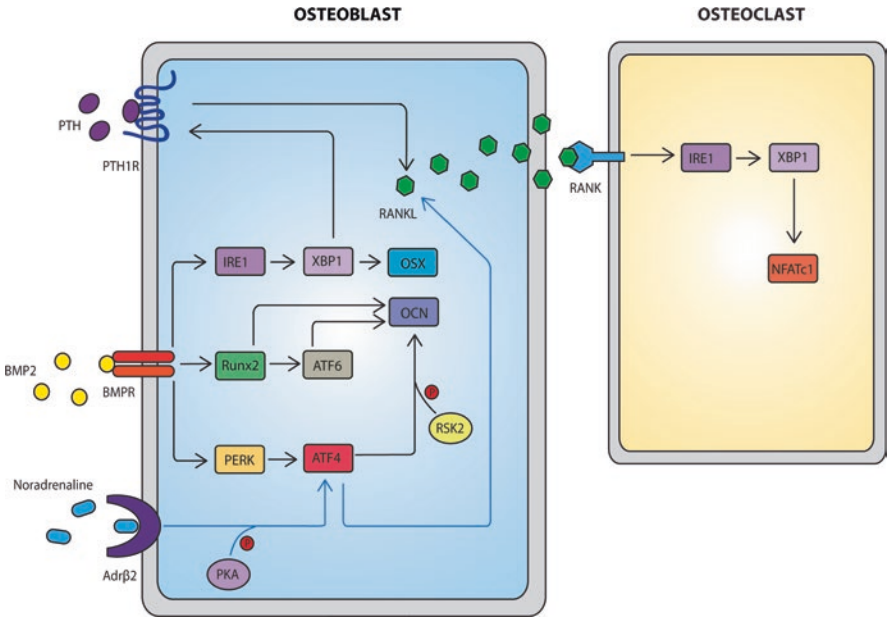

Fig. 2.3 The UPR in osteogenesis. BMP2 signalling induces IRE1-dependent XBP1 splicing. XBP1 is required for expression of Osterix (OSX), a transcription factor driving osteoblast differentiation. IRE1-XBP1 also regulates the transcription of PTH1R in osteoblasts. PTH signalling induces expression of RANKL, an osteoclastogenic cytokine secreted by osteoblasts. RANKL-mediated signalling in osteoclasts further activates IRE1-mediated XBP1 splicing, thus inducing expression of NFATc1 to promote osteoclast differentiation. BMP2 triggers PERK-dependent expression of ATF4, and RSK2-mediated activation of ATF4 facilitates the expression of the hormone osteocalcin (OCN) by osteoblasts. Sympathetic signalling via ß2-adrenergic receptors (Adrβ2) on osteoblasts activates protein kinase A-mediated activation of ATF4. BMP2-mediated signalling also induces ATF6 expression in a Runx2-dependent manner. In cooperation with Runx2 and ATF4, ATF6 regulates OCN expression

osteoblast markers *osterix and osteocalcin* [100]. The transcription factor *osterix* appears to be a direct target of XBP1 (Fig. 2.3), and its expression in IRE1-deficient mouse embryonic fibroblasts partially rescues BMP2-induced alkaline phosphate expression. These findings implicate XBP1 in the late stage of osteoblast differentiation.

In view of this role in osteoblast development, it was surprising that deletion of IRE1 in bone marrow cells led to an increase in bone mass [101]. This occurred because IRE1 and XBP1 also regulate expression of nuclear factor of activated T cells cytoplasmic 1 (NFATc1), a key factor in the development of bone-resorbing osteoclasts (Fig. 2.3) [101]. As a result, loss of IRE1α is associated with impaired osteoclast differentiation. IRE1α becomes activated transiently during osteoclastogenesis, possibly through calcium loss from the ER via the inositol 1,4,5-trisphosphate receptors 2 and 3 since their blockade prevents activation of IRE1α. Furthermore, XBP1 has been shown to regulate osteoclastogenesis by regulating the transcription

of parathyroid hormone (PTH)/PTH-related peptide receptor (PTH1R) in osteoblasts (Fig. 2.3). PTH signalling has been shown to induce expression of the Receptor activator of NF-κB ligand (*Rankl*) gene, which encodes an osteoclastogenic cytokine secreted by osteoblasts [102, 103]. Gene silencing of *Ire1*α and *Xbp1* was shown to block the BMP2-mediated increase in *Pth1r* transcription in mouse embryonic fibroblasts, and XBP1 was later shown to regulate *Pth1r* transcription directly [104]. In addition, gene silencing of *Xbp1* suppressed PTH-induced *Rankl* expression in primary osteoblasts and thereby abolished osteoclast formation in an *in vitro* model of osteoclastogenesis [104].

XBP1 also has a more subtle role in the development of cartilage. Although a report suggesting that XBP1s associates with Runt-related transcription factor 2 (RUNX2) to regulate chondrocyte growth was retracted [105], a later paper identified phenotypes in mice with cartilage-specific XBP1 inactivation (*Xbp1CartΔEx2*) [106]. Ablation of XBP1 did not prevent chondrocyte hypertrophy *in vivo,* but instead led to chondrodysplasia with shortening of endochondral bones and delayed endochondral ossification during development. However, this phenotype had resolved by the time of skeletal maturity suggesting that, unlike in bone, loss of XBP1 in cartilage can be compensated.

Epithelial-to-Mesenchymal Transition

Epithelial-to-mesenchymal transition (EMT) is a frequent and important process during embryonic development because of the requirement to reorganize germ layers and tissues. EMT is de-differentiative process during which epithelial cells lose their polarity and adhesive properties and revert to mesenchymal cells with invasive and migratory properties. Its importance is not restricted to early embryonic morphogenesis since it also fulfils an important patterning function by generating novel tissue juxtapositions that will facilitate further patterning events. In pathology, EMT also plays an important role in many human diseases. In response to a variety of stressful stimuli, secondary epithelium in adult tissues can undergo EMT resulting in fibroblast production and fibrogenesis, while epithelial tumours undergo EMT prior to metastasis [107–110].

Emerging evidence suggests that ER stress promotes EMT and fibrosis [111–114]. Treatment of alveolar cells with tunicamycin (a drug that causes ER stress throught selective inhibition of N-glycosylation) or the chronic expression of mutant surfactant protein C have both been shown to induce EMT [114]. Similarly, thyroid epithelial cells exposed to ER stress-inducing drugs exhibited EMT [111]. This de-differentiation process seems to be mediated through Smad2/3 and Src kinase signalling, as specific inhibition of these pathways blocked EMT and so maintained the epithelial phenotype [111, 114]. While the precise mechanism linking the UPR to Smad and Src activation remains unclear, siRNA targeting of IRE1, but not PERK or ATF6, was found to attenuate tunicamycin-induced Smad2 and Src phosphorylation [114]. Although these studies did not examine the physiological relevance of

the UPR in EMT, it is plausible that the IRE1-XBP1 axis may also contribute to EMT during embryonic development.

PERK-eIF2α-ATF4 Axis in Development

Pancreas

The importance of PERK signalling during pancreatic development was first highlighted by a genetic disease causing early-onset diabetes. PERK deficiency leads to Wolcott-Rallison syndrome, an autosomal recessive disorder, characterized by early-onset non-autoimmune diabetes associated with exocrine pancreas atrophy, skeletal dysplasia, and growth retardation [115, 116]. Classical genetic analysis mapped the causative mutations of this condition to the *EIF2AK3* gene, which encodes PERK [117]. Studies using *Perk*$^{-/-}$ mice showed that loss of PERK led to severe hyperglycaemia in 4-week-old animals due to selective degeneration of insulin secreting β-cells [49]. A subsequent reduction of islet size was also detected, followed by a dramatic loss of islet numbers by 6 weeks of age [49, 118]. *Perk*$^{-/-}$ mice showed aberrant proinsulin accumulation and increased insulin biosynthesis owing to an inability to limit secretory protein synthesis, which is necessary to protect the cell from developing ER stress [49, 118]. Accumulation of proinsulin in the ER of *Perk*$^{-/-}$ β-cells was initially thought to be due to derepression of protein synthesis and protein overload. Increasing evidence, however, suggests that ablation of PERK in beta cells results in proinsulin aggregation, induction of chaperone expression, and defects in ERAD and trafficking in the absence of ER stress responses [119, 120]. More recently, selective antagonism of PERK using a small molecule inhibitor was shown to reduce proinsulin synthesis, while promoting proinsulin aggregation through perturbation of ER calcium dynamics and chaperone activity [121]. These findings suggest that altered ER chaperone activity and expression, rather than derepression of proinsulin synthesis, may link loss of PERK function to β-cell dysfunction and the development of neonatal diabetes.

Mice homozygous for the *eIF2α*^{S51A} allele, which cannot be phosphorylated by PERK, develop severe hypoglycaemia and die within 18 h of birth [51]. Neonatal mice normally develop a transient hypoglycaemia that is compensated for by mobilizing glycogen stores that sustain the pup until it can begin to feed [122]. However, homozygous *eIF2α*^{S51A} neonates lack glycogen stores at birth owing to embryonic insulin insufficiency [51]. As *Perk*$^{-/-}$ mice die of hyperglycaemia after several weeks [49], while homozygous *eIF2α*^{S51A} pups die rapidly of hypoglycaemia [51], this difference in phenotype suggests the involvement of addition eIF2α kinases during development and the regulation of glucose homeostatus early in life.

Tissue-specific knockout studies have shown that β-cells require expression of PERK in order to develop during the fetal and early post-natal periods [123]. β-cells are generated in the mouse by differentiation of endocrine precursor cells between

E13.5 and E16.5, while β-cell expansion occurs during the late fetal and neonatal periods. Careful analysis of tissue-specific *Perk*$^{-/-}$ mice revealed lower β-cell mass at birth that could be attributed to reduced β-cell proliferation rather than increased apoptosis. Furthermore, transcriptional profiling identified many genes involved in cell cycle progression and proliferation, including cyclin A and CDK1, to be reduced in *Perk*$^{-/-}$ islets [123]. Acute ablation of *Perk* in a rat β-cell line also resulted in reduced expression of β-cells markers, reduced insulin secretion, and an overall reduction in β-cell proliferation [124]

The mechanism by which eIF2α phosphorylation affects β-cell development remains to be fully understood, but may involve regulation of signalling by insulin-like growth factor-1 (IGF-1). IGF-1 is known to be trophic during β-cell development via effects mediated by insulin receptor substrate-2 (IRS-2). Consequently, both IGF-1 receptor-deficient and IRS-2-deficient mice show reduced β-cell mass at birth with increased expression of proapoptotic proteins [125]. *Perk*$^{-/-}$ mice have reduced pancreatic expression of *Igf1* mRNA and low circulating IGF-1 levels during the neonatal period suggesting that eIF2α may be necessary to promote IGF-1 synthesis [126].

Osteogenesis

An early clue that ATF4 is important in bone development came from Coffin-Lowry syndrome, an X-linked disorder causing mental retardation and progressive skeletal abnormalities [54]. The disease is caused by mutations of ribosomal S6 protein kinase 2 (RSK2), a growth factor-regulated serine/threonine protein kinase [127]. *In vitro*, RSK2 phosphorylates the cyclic AMP response element binding protein (CREB) and is necessary for growth factor-stimulated transcription of the immediate-early gene c-Fos [128, 129]. However, while *Rsk2*-deficient mice recapitulate the delayed mineralization and decreased bone mass seen in Coffin-Lowry syndrome individuals [54], they fail to show any alterations in CREB phosphorylation or c-Fos expression [130]. *In vitro* kinase assays with various leucine-zipper proteins identified ATF4 as a more efficient RSK2 substrate, and ATF4 phosphorylation was found to be decreased in osteoblasts derived from *Rsk2*-deficient mice [54]. Subsequent analysis revealed that *Atf4*-deficient mice displayed a substantial reduction of bone mineralization throughout life [54]. Molecular analysis demonstrated that *Atf4*-deficient animals had normal levels of RUNX2 and osterix, which are required for the initiation of osteoblast differentiation from mesenchymal stem cells [54]. However, expression of bone sialoprotein and osteocalcin, markers for terminal osteoblast differentiation, were markedly reduced [54]. Expression of a constitutively active form of RSK2 (RSK2-T707A) activated an *Osteocalcin* promoter luciferase reporter containing both osteoblast-specific element (OSE)-1 and OSE2 [131]. This effect required an intact OSE1 element and was abolished in the presence of an ATF4 mutant that could not be phosphorylated by RSK2 [54].

Osteocalcin was found to be a direct target of ATF4 via binding to the OSE1 close to the Runx2 *cis*-acting element, OSE2 [54, 132, 133]. ATF4 interacts synergistically with Runx2 through the nuclear matrix protein Satb2 to induce osteocalcin expression [134]. These findings suggest that phosphorylation of ATF4 by RSK2 enhances its transactivation of target genes including *Osteocalcin* (Fig. 2.3).

ATF4 is also necessary for the regulation of type 1 collagen (Col1A1) synthesis by osteoblasts, but not necessarily at the transcriptional level [54]. *Col1A1* gene expression remains unchanged in *Atf4*-deficient osteoblasts despite a marked reduction of collagen synthesis by *Atf4*$^{-/-}$ osteoblasts [54]. This can be rescued by the addition of nonessential amino acids to the primary culture medium indicating that osteoblasts, like many cells, require ATF4 for efficient amino acid import and tRNA acylation [10].

ATF4 has also been shown to regulate protein secretion via its target CHOP [12]. *Chop*$^{-/-}$ mice showed reduced mRNA levels for type I collagen and osteocalcin in bone and exhibited decreased bone formation rates compared to controls [56]. However, over-expression of CHOP reduced alkaline phosphatase activity in osteoblasts and led to reduced bone mineral density in mice, while *Chop*$^{-/-}$ osteoblasts differentiated more strongly than their wild-type controls *in vitro* [57, 58]. By contrast, endogenous CHOP-induced differentiation of calvarial osteoblasts upon BMP treatment [58]. Thus, it appears that CHOP has a complex role in osteoblast function, both inhibiting differentiation in some settings, but able to promote BMP signalling in a cell-type-specific manner.

In addition to its role in osteoblast function, ATF4 is also required for efficient osteoclast differentiation [103]. The sympathetic nervous system has been shown to play a key role in bone remodelling by regulating *Rankl* expression [103]. A subsequent study has demonstrated that ATF4 is directly responsible for regulating the transactivation of this osteogenic gene in response to sympathetic signalling via β2-adrenergic receptors present on osteoblasts (Fig. 2.3) [103]. Interestingly, sympathetic regulation of ATF4-mediated Rankl expression occurs independently of RSK2, and relies instead on protein kinase A-mediated phosphorylation of ATF4 [103].

ATF4 also plays a key role in the regulation of chondrocyte proliferation and differentiation through Indian Hedgehog (*Ihh*) [55]. *Atf4*-deficient embryos displayed severe cartilage defects with a small and disorganized proliferative zone, reduced proliferation, accelerated hypertrophy, and decreased levels of *Ihh* expression and Hedgehog (Hh) signalling [55]. While Ihh expression in chondrocytes is directly regulated by ATF4, the reactivation of Hh signalling in mouse limb explants can bypass this and correct the cartilage abnormalities in *Atf4*$^{-/-}$ mice [55]. Interestingly, chondrocyte-specific expression of ATF4 is also required for efficient osteoblast differentiation. Conditioned media from *ex vivo* cultures of wild-type ATF4-expressing cartilage, but not *Atf4*$^{-/-}$ cartilage, could reverse osteoblast differentiation defects of *Atf4*$^{-/-}$ mesenchymal stem cells [135]. These effects also appear to be Ihh mediated, as Ihh-blocking antibody abolishes this effect [135].

Although the role of ATF4 in skeletal development is well established, the involvement of PERK is less clear. *PERK* loss-of-function mutations in humans and

mice cause developmental defects including skeletal dysplasia [50, 117]. The bones of *Perk*-deficient mice are severely osteopenic due to a reduction in mature osteoblasts and diminished osteoblast differentiation, just as had been observed in *Atf4*$^{-/-}$ mice [50]. These findings, and the observation that ER stress occurs during normal osteoblast differentiation, implicated the PERK-eIF2α-ATF4 pathway in osteogenesis [61]. The levels of eIF2α phosphorylation and ATF4 protein translation are markedly reduced in *Perk*$^{-/-}$ bone, and expression of the ATF4 targets, osteocalcin and Bsp, are both reduced [136]. Treatment of primary bone cultures with BMP2 triggers ER stress and induces ATF4 levels in a PERK-dependent manner [136]. Accordingly, *Perk*-deficient osteoblasts demonstrated a delay in mineralization, and exhibited decreased alkaline phosphatase activities relative to wild-type cultures [136]. These defects could be largely corrected by the introduction of ATF4 into *Perk*$^{-/-}$ osteoblasts [136].

Recently, hints to the mechanisms linking BMP signalling and eIF2α-ATF4 pathway have come from studies in fruitfly models. eIF2α hyperphosphorylation was found to play a key role in *Drosophila* wing tissue morphogenesis through the modulation of BMP signalling [137]. Genetic manipulations that either increased or reduced phosphorylation of eIF2α in the developing *Drosophila* wing, led to changes in the phosphorylation of MAD, the transcription factors downstream of BMP signalling in the fly (and homologous to the mammalin SMADs). In cultured S2 insect cells, phosphorylation eIF2α attenuated the normal phosphorylation of MAD caused by the addition of BMP receptor ligand Dpp (homologous to BMP4). These antagonistic effects on BMP signalling were mediated both via a direct effect on translation, but also indirectly through the increased expression of Cryptocephal (crc), the *Drosophila* homologue of ATF4 [137–139]. Indeed, over-expression of crc/ATF4 in S2 cells significantly reduced MAD phosphorylation in response to BMP receptor activation. This effect of crc/ATF4 was mediated, at least in part, by the induction of d4E-BP, a regulator of cap-dependent translation known to be a target of crc/ATF4 [137, 140]. While 4E-BP can both attenuate cap-dependent translation and bias translation towards specific mRNAs, the precise mechanism by which 4E-BP inhibits BMP signalling remains to be elucidated.

Intriguingly, in humans two rare subtypes of pulmonary arterial hypertension (PAH) called pulmonary veno occlusive disease (PVOD) and capillary haemangiomatosis were found to be caused by mutations of *EIF2AK4*, which encodes the eIF2α kinase GCN2 [141, 142]. Although most cases of PAH cases are idiopathic, in 70% of familial cases and 20% of sporadic cases, heterozygous germline mutations are identified in the type II BMP receptor (*BMPR2*). Interestingly, *BMPR2* mutations have also been associated with PVOD [143, 144], suggesting that both eIF2α phosphorylation and perturbed BMP signalling can cause this condition. Moreover, a comprehensive analysis of the genetic defects found in apparently sporadic cases of PAH revealed that mutations within the BMP and GCN2 signalling pathways predominate [145]. Precisely why impaired eIF2α phosphorylation should result in PAH is not yet clear, but the striking relationship between eIF2α phosphorylation and BMP signalling disovered in *Drosophila* may have provided an important clue.

Adipogenesis

Adipocyte differentiation is coordinately regulated by a number of transcription factors; CCAAT/enhancer binding proteinβ (C/EBPβ) and C/EBPδ are active during the early stages of adipocyte differentiation and induce expression of the peroxisome proliferator-activated receptor gamma (PPARγ) and C/EBPα [146–149]. PPARγ is a master regulator of adipogenesis, mediating cell cycle exit and inducing the expression of many adipocyte genes [150, 151]. C/EBPα cooperates with PPARγ to induce expression of additional target genes and acts as a positive regulator of PPARγ [151].

CHOP, itself a C/EBP family protein, acts to oppose C/EBP activity during adipogenesis [152]. While CHOP has strong sequence homology with the other C/EBP family members, two proline substitutions in the basic region of its DNA-binding domain prevent binding to the C/EBP cognate DNA enhancer element [152]. Given its ability to form heterodimers with C/EBP family members, CHOP was believed to act as a dominant-negative inhibitor of C/EBP activity [152]. Indeed, ectopic expression of CHOP was shown to attenuate adipocytic differentiation of 3T3-L1 cells [153]. Furthermore, CHOP-expressing cells exposed to a differentiation cocktail were unable to induce C/EBPα expression, and C/EBPβ levels dropped relative to wild-type cells, while C/EBPδ expression remained unchanged [153]. Subsequent work showed endogenous CHOP to interact with C/EBPβ and delay PPARγ and C/EBPα expression [154].

In keeping with its role as a suppressor of adipocyte differentiation, CHOP is expressed in preadipocytes, but is downregulated once the clonal expansions preceding adipogenesis begins [155]. CHOP remains suppressed for the first few days of differentiation, but returns during the end stages of the adipogenic programme [155]. Thus, CHOP appears to play a role in terminating the differentiation process once key events have been initiated. Indeed, $Chop^{-/-}$ mice gained more fat mass than wild-type mice when fed a high fat diet, and combined $Chop^{-/-}$ and leptin receptor-deficient ($Lepr^{db/db}$) mice showed a significant increase in body fat mass without affecting adipocyte size [53].

More recent studies examining the role of ER stress in adipogenesis have demonstrated that the pattern of eIF2α phosphorylation during adipocyte differentiation closely mirrors the induction of CHOP. During differentiation of 3T3-L1 into adipocytes, phosphorylated and total eIF2α were both reduced at days 1–2 and increased again during days 3–7 [53, 156]. Moreover, treatment with tunicamycin to induce ER stress inhibited adipogenesis in the 3T3-L1 differentiation model [53]. Similarly, pre-emptive phosphorylation of eIF2α by drug-activatable Fv2E-PERK inhibited adipogenic differentiation of 3T3-L1 cells [53]. Conversely, homozygous knockin mouse embryonic fibroblasts, expressing only the non-phosphorylatable $eIF2\alpha^{S51A}$, showed significantly enhanced adipogenesis, while $eIF2\alpha^{S51A/+}$ heterozygous mice developed increased obesity and adipocyte number on a high fat diet [53].

ATF6 in Development

Mice deficient in either *Atf6α* or *Atf6β* develop normally, while *Atf6α/β* double knockout is embryonic lethal [59]. This suggests functional redundancy between the isoforms, and a key role for ATF6 in early development. In the model fish, *Oryzias latipes, Atf6α/β* double knockout is also embryonic lethal but unlike the mouse, this animal is amenable to study during gestation [157]. The level of ER stress was monitored during early embryonic development using a BiP reporter, and ATF6α- and ATF6β-mediated induction of BiP was detected primarily in the brain, otic vesicles, and notochord. Microscopic analysis demonstrated a severely degenerated notochord in *ATF6α/β* double knockout embryos [157]. This phenotype could be partially rescued by the over-expression of BiP, implicating failure of ER proteostasis [157]. Moreover, knockdown of the α1 chain of type VIII collagen, one of the extracellular matrix proteins secreted in large amounts upon notochord formation, reduced this ER stress [157]. Collectively, these findings suggest that the role for ATF6 in the physiological development of fish relates, at least partially, to its ability to increase chaperone levels in response to increased ER load.

Myoblasts

During normal development, a large percentage of differentiating myoblasts undergo apoptosis [158]. Indeed, myogenic differentiation has been shown to depend on the activity of the key apoptotic protease, caspase-3 [159]. The putative ER-stress selective caspase-12 is also activated in differentiating myoblasts *in vivo* and *in vitro* [160]. It must be noted, however, that the relevance of caspase-12 to ER-induced apoptosis is questionable as it has acquired deleterious mutations in most humans, and so most people are effectively caspase-12 null [161].

ATF6, but none of the other ER stress sensors, is activated in apoptotic myoblasts, and inhibition of a protease involved in ATF6 activation blocked apoptosis and myotube formation in myogenic culture models [160, 162]. While treatment of a myoblast cell line (C2C12) with drugs that cause ER stress-induced apoptosis of some cells, the surviving cells showed enhanced cell fusion and eventually differentiated into contracting myofibers five- to tenfold larger than untreated control cells [163]. Although an *in vitro* model, these findings echo the action of ATF6-driven signals *in vivo*. Interestingly, loss of ATF6 compromises muscle recovery after acute exercise, and increased intolerance to exercise in an *in vivo* model of adaptation to exercise [164].

Osteogenesis

Recently, a possible role for ATF6 in osteoblast differentiation was identified. Treatment of the osteoblastic cell line MC3T3-E1 with BMP2 was shown to induce expression and activation of ATF6 [165]. By contrast, BMP2 treatment failed to induce ATF6 expression in Runx2-deficient primary osteoblasts, but Runx2 overexpression was able to rescue BMP2-induced ATF6 expression [165]. BMP2 increased ATF6 transcription by promoting Runx2 binding to an OSE2-like element in the ATF6 promoter. Moreover, ATF6 was shown to bind directly to the osteocalcin promoter and regulate its expression (Fig. 2.3) [165]. Interestingly, Runx2 and ATF4-mediated osteocalcin expression were enhanced upon ATF6 co-expression, suggesting a possible cooperative role for ATF6 in osteocalcin expression [165]. Although *Atf6*$^{-/-}$ mouse studies have been hampered by embryonic lethality of the double mutant, matrix mineralization assays *in vitro* suggest that BMP2-induced mineralization is, in part, dependent upon ATF6 expression [165]. ATF6 has also been implicated in odontoblastic differentiation [166]. It was suggested that ATF6 induces dentin sialophosphoprotein and dentin matrix protein 1. Additionally, as in the bone studies, inhibition of ATF6 decreased alkaline phosphatase activity and mineralization.

Photoreceptors

ATF6 has been implicated in foveal development and cone function. Achromatopsia is an autosomal recessive disorder of colour blindness, photophobia, nystagmus, and severely reduced visual acuity. Homozygosity mapping, combined with whole-exome and candidate gene sequencing, identified mutations in the *ATF6α* gene in several families with this condition [60]. Affected patients exhibited foveal hypoplasia and degeneration of the cone photoreceptor layer. A similar phenotype had not been noted previously in *Atf6α*$^{-/-}$ mice because the retina of young animals appeared normal. However, following this clinical observation in humans, older mice were studied and observed to develop marked degeneration of photoreceptors [60]. A subsequent study examining a patient diagnosed with early-onset photoreceptor degeneration also identified biallelic loss-of-function mutations in the *ATF6* gene [167]. A recent study has shown the ATF6 can function to suppress the pluripotency of stem cells, and this ability is deficient in mutants of ATF6 associated with devopmental defects of vision [168]. While explaining the failure of neuroretina development, these insights offer a potential new avenue to generate mesoderm from stem cells for experimental and potentially therapeutic use.

ATF6 Paralogues in Development

Other members of the ATF6 family have also been implicated in development. The ER membrane-bound bZIP transcription factor OASIS (old astrocyte specifically induced substance) shares significant structural similarities with ATF6 and has been described as an ER-stress transducer [169–171]. OASIS-deficient mice display severe osteopenia characterized by a marked decrease in bone density and spontaneous fractures [61]. Subsequent genetic analyses have demonstrated that collagen 1a1, a major component in bone tissue, is a direct transcriptional target of OASIS.

OASIS is also preferentially expressed in astrocytes in the central nervous system [170] and has been shown to regulate the differentiation of neural precursor cells into astrocytes [62]. It directly regulates expression of the transcription factor GCM1 (glial cells missing 1), which is necessary in *Drosophila* for astrocyte differentiation [62, 172]. Over-expression of GCM1 in OASIS-deficient neural precursor cells improved their differentiation into astrocytes by accelerating the promoter demethylation of a key astrocyte marker, glial fibrillary acidic protein (GFAP) [62].

Conclusion

Although once thought to serve primarily as a stress response, there is now much evidence highlighting the involvement of the UPR in normal development and differentiation both of secretory and non-secretory lineages. In this context, the UPR serves to anticipate the increased demand for ER protein folding as tissues differentiate, while also participating directly in developmental signalling. These processes appear to be subverted by some cancers to aid adaption to highly secretory phenotypes, most notably in myeloma, but might also drive progression of disease, for example, during epithelial to mesenchyme transition.

References

1. Chambers JE, Marciniak SJ. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 2. Protein misfolding and ER stress. Am J Physiol Cell Physiol. 2014;307(8):C657–70.
2. Derman AI, Beckwith J. Escherichia coli alkaline phosphatase fails to acquire disulfide bonds when retained in the cytoplasm. J Bacteriol. 1991;173(23):7719–22.
3. Lippincott-Schwartz J, Bonifacino JS, Yuan LC, Klausner RD. Degradation from the endoplasmic reticulum: disposing of newly synthesized proteins. Cell. 1988;54(2):209–20.
4. Koo EH, Lansbury PT Jr, Kelly JW. Amyloid diseases: abnormal protein aggregation in neurodegeneration. Proc Natl Acad Sci U S A. 1999;96(18):9989–90.
5. Roussel BD, Kruppa AJ, Miranda E, Crowther DC, Lomas DA, Marciniak SJ. Endoplasmic reticulum dysfunction in neurological disease. Lancet Neurol. 2013;12(1):105–18.

6. Clarke HJ, Chambers JE, Liniker E, Marciniak SJ. Endoplasmic reticulum stress in malignancy. Cancer Cell. 2014;25(5):563–73.
7. Saliba RS, Munro PM, Luthert PJ, Cheetham ME. The cellular fate of mutant rhodopsin: quality control, degradation and aggresome formation. J Cell Sci. 2002;115(Pt 14):2907–18.
8. Thomas SE, Dalton L, Malzer E, Marciniak SJ. Unravelling the story of protein misfolding in diabetes mellitus. World J Diabetes. 2011;2(7):114–8.
9. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature. 1999;397(6716):271–4.
10. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell. 2003;11(3):619–33.
11. Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, et al. Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. J Clin Invest. 2002;109(4):525–32.
12. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, et al. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev. 2004;18(24):3066–77.
13. Tirasophon W, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes Dev. 1998;12(12):1812–24.
14. Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D. Cloning of mammalian Ire1 reveals diversity in the ER stress responses. EMBO J. 1998;17(19):5708–17.
15. Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, Kimata Y, et al. Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. Nat Cell Biol. 2001;3(2):158–64.
16. Tsuru A, Fujimoto N, Takahashi S, Saito M, Nakamura D, Iwano M, et al. Negative feedback by IRE1beta optimizes mucin production in goblet cells. Proc Natl Acad Sci U S A. 2013;110(8):2864–9.
17. Martino MB, Jones L, Brighton B, Ehre C, Abdulah L, Davis CW, et al. The ER stress transducer IRE1beta is required for airway epithelial mucin production. Mucosal Immunol. 2013;6(3):639–54.
18. Credle JJ, Finer-Moore JS, Papa FR, Stroud RM, Walter P. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. Proc Natl Acad Sci U S A. 2005;102(52):18773–84.
19. Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F. Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. Cell. 2008;132(1):89–100.
20. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature. 2002;415(6867):92–6.
21. Lu Y, Liang FX, Wang X. A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. Mol Cell. 2014;55(5):758–70.
22. Jurkin J, Henkel T, Nielsen AF, Minnich M, Popow J, Kaufmann T, et al. The mammalian tRNA ligase complex mediates splicing of XBP1 mRNA and controls antibody secretion in plasma cells. EMBO J. 2014;33(24):2922–36.
23. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol. 2003;23(21):7448–59.
24. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol. 2006;26(24):9220–31.
25. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol. 2009;186(3):323–31.
26. Oikawa D, Tokuda M, Hosoda A, Iwawaki T. Identification of a consensus element recognized and cleaved by IRE1 alpha. Nucleic Acids Res. 2010;38(18):6265–73.

27. Hollien J, Weissman JS. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. Science. 2006;313(5783):104–7.
28. Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, et al. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell. 2009;138(3):562–75.
29. Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, et al. The unfolded protein response signals through high-order assembly of Ire1. Nature. 2009;457(7230):687–93.
30. Marciniak SJ, Garcia-Bonilla L, Hu J, Harding HP, Ron D. Activation-dependent substrate recruitment by the eukaryotic translation initiation factor 2 kinase PERK. J Cell Biol. 2006;172(2):201–9.
31. Pain VM. Initiation of protein synthesis in mammalian cells. Biochem J. 1986;235(3):625–37.
32. Lu PD, Harding HP, Ron D. Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. J Cell Biol. 2004;167(1):27–33.
33. Vattem KM, Wek RC. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc Natl Acad Sci U S A. 2004;101(31):11269–74.
34. Wang XZ, Lawson B, Brewer JW, Zinszner H, Sanjay A, Mi LJ, et al. Signals from the stressed endoplasmic reticulum induce C/EBP-homologous protein (CHOP/GADD153). Mol Cell Biol. 1996;16(8):4273–80.
35. Connor JH, Weiser DC, Li S, Hallenbeck JM, Shenolikar S. Growth arrest and DNA damage-inducible protein GADD34 assembles a novel signaling complex containing protein phosphatase 1 and inhibitor 1. Mol Cell Biol. 2001;21(20):6841–50.
36. Ma Y, Hendershot LM. Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. J Biol Chem. 2003;278(37):34864–73.
37. Novoa I, Zhang Y, Zeng H, Jungreis R, Harding HP, Ron D. Stress-induced gene expression requires programmed recovery from translational repression. EMBO J. 2003;22(5):1180–7.
38. Shen J, Chen X, Hendershot L, Prywes R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Developmental Cell. 2002;3(1):99–111.
39. Nadanaka S, Okada T, Yoshida H, Mori K. Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. Mol Cell Biol. 2007;27(3):1027–43.
40. Shen J, Prywes R. Dependence of site-2 protease cleavage of ATF6 on prior site-1 protease digestion is determined by the size of the luminal domain of ATF6. J Biol Chem. 2004;279(41):43046–51.
41. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M, et al. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol Cell Biol. 2000;20(18):6755–67.
42. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001;107(7):881–91.
43. Zhang K, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. The unfolded protein response sensor IRE1alpha is required at 2 distinct steps in B cell lymphopoiesis. J Clin Invest. 2005;115(2):268–81.
44. Iwawaki T, Akai R, Yamanaka S, Kohno K. Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. Proc Natl Acad Sci U S A. 2009;106(39):16657–62.
45. Reimold AM, Etkin A, Clauss I, Perkins A, Friend DS, Zhang J, et al. An essential role in liver development for transcription factor XBP-1. Genes Dev. 2000;14(2):152–7.
46. Lee AH, Chu GC, Iwakoshi NN, Glimcher LH. XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. EMBO J. 2005;24(24):4368–80.
47. Hu CC, Dougan SK, McGehee AM, Love JC, Ploegh HL. XBP-1 regulates signal transduction, transcription factors and bone marrow colonization in B cells. EMBO J. 2009;28(11):1624–36.

48. Iwakoshi NN, Pypaert M, Glimcher LH. The transcription factor XBP-1 is essential for the development and survival of dendritic cells. J Exp Med. 2007;204(10):2267–75.
49. Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, et al. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. Mol Cell. 2001;7(6):1153–63.
50. Wei J, Sheng X, Feng D, McGrath B, Cavener DR. PERK is essential for neonatal skeletal development to regulate osteoblast proliferation and differentiation. J Cell Physiol. 2008;217(3):693–707.
51. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, et al. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol Cell. 2001;7(6):1165–76.
52. Scheuner D, Vander Mierde D, Song B, Flamez D, Creemers JW, Tsukamoto K, et al. Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. Nat Med. 2005;11(7):757–64.
53. Han J, Murthy R, Wood B, Song B, Wang S, Sun B, et al. ER stress signalling through eIF2alpha and CHOP, but not IRE1alpha, attenuates adipogenesis in mice. Diabetologia. 2013;56(4):911–24.
54. Yang X, Matsuda K, Bialek P, Jacquot S, Masuoka HC, Schinke T, et al. ATF4 is a substrate of RSK2 and an essential regulator of osteoblast biology; implication for Coffin-Lowry syndrome. Cell. 2004;117(3):387–98.
55. Wang W, Lian N, Li L, Moss HE, Wang W, Perrien DS, et al. Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating Ihh transcription. Development. 2009;136(24):4143–53.
56. Pereira RC, Stadmeyer L, Marciniak SJ, Ron D, Canalis E. C/EBP homologous protein is necessary for normal osteoblastic function. J Cell Biochem. 2006;97(3):633–40.
57. Pereira RC, Stadmeyer LE, Smith DL, Rydziel S, Canalis E. CCAAT/enhancer-binding protein homologous protein (CHOP) decreases bone formation and causes osteopenia. Bone. 2007;40(3):619–26.
58. Shirakawa K, Maeda S, Gotoh T, Hayashi M, Shinomiya K, Ehata S, et al. CCAAT/enhancer-binding protein homologous protein (CHOP) regulates osteoblast differentiation. Mol Cell Biol. 2006;26(16):6105–16.
59. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. Dev Cell. 2007;13(3):365–76.
60. Kohl S, Zobor D, Chiang WC, Weisschuh N, Staller J, Gonzalez Menendez I, et al. Mutations in the unfolded protein response regulator ATF6 cause the cone dysfunction disorder achromatopsia. Nat Genet. 2015;47(7):757–65.
61. Murakami T, Saito A, Hino S, Kondo S, Kanemoto S, Chihara K, et al. Signalling mediated by the endoplasmic reticulum stress transducer OASIS is involved in bone formation. Nat Cell Biol. 2009;11(10):1205–11.
62. Saito A, Kanemoto S, Kawasaki N, Asada R, Iwamoto H, Oki M, et al. Unfolded protein response, activated by OASIS family transcription factors, promotes astrocyte differentiation. Nat Commun. 2012;3:967.
63. Malzer E, Szajewska-Skuta M, Dalton LE, Thomas SE, Hu N, Skaer H, et al. Coordinate regulation of eIF2alpha phosphorylation by PPP1R15 and GCN2 is required during Drosophila development. J Cell Sci. 2013;126(Pt 6):1406–15.
64. Urano F, Bertolotti A, Ron D. IRE1 and efferent signaling from the endoplasmic reticulum. J Cell Sci. 2000;113(Pt 21):3697–702.
65. Kozak KR, Abbott B, Hankinson O. ARNT-deficient mice and placental differentiation. Dev Biol. 1997;191(2):297–305.
66. Oikawa D, Akai R, Iwawaki T. Positive contribution of the IRE1alpha-XBP1 pathway to placental expression of CEA family genes. FEBS Lett. 2010;584(5):1066–70.

67. Ergun S, Kilik N, Ziegeler G, Hansen A, Nollau P, Gotze J, et al. CEA-related cell adhesion molecule 1: a potent angiogenic factor and a major effector of vascular endothelial growth factor. Mol Cell. 2000;5(2):311–20.
68. Clauss IM, Gravallese EM, Darling JM, Shapiro F, Glimcher MJ, Glimcher LH. In situ hybridization studies suggest a role for the basic region-leucine zipper protein hXBP-1 in exocrine gland and skeletal development during mouse embryogenesis. Dev Dyn. 1993;197(2):146–56.
69. Hess DA, Humphrey SE, Ishibashi J, Damsz B, Lee AH, Glimcher LH, et al. Extensive pancreas regeneration following acinar-specific disruption of Xbp1 in mice. Gastroenterology. 2011;141(4):1463–72.
70. Desilva MG, Lu J, Donadel G, Modi WS, Xie H, Notkins AL, et al. Characterization and chromosomal localization of a new protein disulfide isomerase, PDIp, highly expressed in human pancreas. DNA Cell Biol. 1996;15(1):9–16.
71. Volkmer J, Guth S, Nastainczyk W, Knippel P, Klappa P, Gnau V, et al. Pancreas specific protein disulfide isomerase, PDIp, is in transient contact with secretory proteins during late stages of translocation. FEBS Lett. 1997;406(3):291–5.
72. Rapoport TA, Jungnickel B, Kutay U. Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. Annu Rev Biochem. 1996;65:271–303.
73. Hosokawa N, Wada I, Hasegawa K, Yorihuzi T, Tremblay LO, Herscovics A, et al. A novel ER alpha-mannosidase-like protein accelerates ER-associated degradation. EMBO Rep. 2001;2(5):415–22.
74. Wilkinson B, Gilbert HF. Protein disulfide isomerase. Biochim Biophys Acta. 2004;1699(1–2):35–44.
75. Pin CL, Rukstalis JM, Johnson C, Konieczny SF. The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity. J Cell Biol. 2001;155(4):519–30.
76. Tian X, Jin RU, Bredemeyer AJ, Oates EJ, Blazewska KM, McKenna CE, et al. RAB26 and RAB3D are direct transcriptional targets of MIST1 that regulate exocrine granule maturation. Mol Cell Biol. 2010;30(5):1269–84.
77. Acosta-Alvear D, Zhou Y, Blais A, Tsikitis M, Lents NH, Arias C, et al. XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. Mol Cell. 2007;27(1):53–66.
78. Huh WJ, Esen E, Geahlen JH, Bredemeyer AJ, Lee AH, Shi G, et al. XBP1 controls maturation of gastric zymogenic cells by induction of MIST1 and expansion of the rough endoplasmic reticulum. Gastroenterology. 2010;139(6):2038–49.
79. Bredemeyer AJ, Geahlen JH, Weis VG, Huh WJ, Zinselmeyer BH, Srivatsan S, et al. The gastric epithelial progenitor cell niche and differentiation of the zymogenic (chief) cell lineage. Dev Biol. 2009;325(1):211–24.
80. Ramsey VG, Doherty JM, Chen CC, Stappenbeck TS, Konieczny SF, Mills JC. The maturation of mucus-secreting gastric epithelial progenitors into digestive-enzyme secreting zymogenic cells requires Mist1. Development. 2007;134(1):211–22.
81. Lennerz JK, Kim SH, Oates EL, Huh WJ, Doherty JM, Tian X, et al. The transcription factor MIST1 is a novel human gastric chief cell marker whose expression is lost in metaplasia, dysplasia, and carcinoma. Am J Pathol. 2010;177(3):1514–33.
82. Wen XY, Stewart AK, Sooknanan RR, Henderson G, Hawley TS, Reimold AM, et al. Identification of c-myc promoter-binding protein and X-box binding protein 1 as interleukin-6 target genes in human multiple myeloma cells. Int J Oncol. 1999;15(1):173–8.
83. Gass JN, Gifford NM, Brewer JW. Activation of an unfolded protein response during differentiation of antibody-secreting B cells. J Biol Chem. 2002;277(50):49047–54.
84. Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravallese EM, et al. Plasma cell differentiation requires the transcription factor XBP-1. Nature. 2001;412(6844):300–7.
85. Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat Immunol. 2003;4(4):321–9.

86. Skalet AH, Isler JA, King LB, Harding HP, Ron D, Monroe JG. Rapid B cell receptor-induced unfolded protein response in nonsecretory B cells correlates with pro- versus antiapoptotic cell fate. J Biol Chem. 2005;280(48):39762–71.
87. Todd DJ, McHeyzer-Williams LJ, Kowal C, Lee AH, Volpe BT, Diamond B, et al. XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. J Exp Med. 2009;206(10):2151–9.
88. Taubenheim N, Tarlinton DM, Crawford S, Corcoran LM, Hodgkin PD, Nutt SL. High rate of antibody secretion is not integral to plasma cell differentiation as revealed by XBP-1 deficiency. J Immunol. 2012;189(7):3328–38.
89. Calame KL, Lin KI, Tunyaplin C. Regulatory mechanisms that determine the development and function of plasma cells. Annu Rev Immunol. 2003;21:205–30.
90. Reimold AM, Ponath PD, Li YS, Hardy RR, David CS, Strominger JL, et al. Transcription factor B cell lineage-specific activator protein regulates the gene for human X-box binding protein 1. J Exp Med. 1996;183(2):393–401.
91. Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, Staudt LM. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. Immunity. 2000;13(2):199–212.
92. Shaffer AL, Lin KI, Kuo TC, Yu X, Hurt EM, Rosenwald A, et al. Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity. 2002;17(1):51–62.
93. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, Qian SB, Zhao H, et al. XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. Immunity. 2004;21(1):81–93.
94. Klein U, Casola S, Cattoretti G, Shen Q, Lia M, Mo T, et al. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nat Immunol. 2006;7(7):773–82.
95. Bagratuni T, Wu P, Gonzalez de Castro D, Davenport EL, Dickens NJ, Walker BA, et al. XBP1s levels are implicated in the biology and outcome of myeloma mediating different clinical outcomes to thalidomide-based treatments. Blood. 2010;116(2):250–3.
96. Mimura N, Fulciniti M, Gorgun G, Tai YT, Cirstea D, Santo L, et al. Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. Blood. 2012;119(24):5772–81.
97. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol. 2005;23:275–306.
98. Osorio F, Tavernier SJ, Hoffmann E, Saeys Y, Martens L, Vetters J, et al. The unfolded-protein-response sensor IRE-1alpha regulates the function of CD8alpha+ dendritic cells. Nat Immunol. 2014;15(3):248–57.
99. Onai N, Obata-Onai A, Tussiwand R, Lanzavecchia A, Manz MG. Activation of the Flt3 signal transduction cascade rescues and enhances type I interferon-producing and dendritic cell development. J Exp Med. 2006;203(1):227–38.
100. Tohmonda T, Miyauchi Y, Ghosh R, Yoda M, Uchikawa S, Takito J, et al. The IRE1alpha-XBP1 pathway is essential for osteoblast differentiation through promoting transcription of Osterix. EMBO Rep. 2011;12(5):451–7.
101. Tohmonda T, Yoda M, Iwawaki T, Matsumoto M, Nakamura M, Mikoshiba K, et al. IRE1alpha/XBP1-mediated branch of the unfolded protein response regulates osteoclastogenesis. J Clin Invest. 2015;125(8):3269–79.
102. Kondo H, Guo J, Bringhurst FR. Cyclic adenosine monophosphate/protein kinase A mediates parathyroid hormone/parathyroid hormone-related protein receptor regulation of osteoclastogenesis and expression of RANKL and osteoprotegerin mRNAs by marrow stromal cells. J Bone Miner Res. 2002;17(9):1667–79.
103. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, et al. Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature. 2005;434(7032):514–20.
104. Tohmonda T, Yoda M, Mizuochi H, Morioka H, Matsumoto M, Urano F, et al. The IRE1alpha-XBP1 pathway positively regulates parathyroid hormone (PTH)/PTH-related

peptide receptor expression and is involved in pth-induced osteoclastogenesis. J Biol Chem. 2013;288(3):1691–5.
105. Liu Y, Zhou J, Zhao W, Li X, Jiang R, Liu C, et al. XBP1S associates with RUNX2 and regulates chondrocyte hypertrophy. J Biol Chem. 2012;287(41):34500–13.
106. Cameron TL, Gresshoff IL, Bell KM, Pirog KA, Sampurno L, Hartley CL, et al. Cartilage-specific ablation of XBP1 signaling in mouse results in a chondrodysplasia characterized by reduced chondrocyte proliferation and delayed cartilage maturation and mineralization. Osteoarthritis Cartilage. 2015;23(4):661–70.
107. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest. 2002;110(3):341–50.
108. Desmouliere A. Factors influencing myofibroblast differentiation during wound healing and fibrosis. Cell Biol Int. 1995;19(5):471–6.
109. Lester RD, Jo M, Montel V, Takimoto S, Gonias SL. uPAR induces epithelial-mesenchymal transition in hypoxic breast cancer cells. J Cell Biol. 2007;178(3):425–36.
110. Guarino M, Micheli P, Pallotti F, Giordano F. Pathological relevance of epithelial and mesenchymal phenotype plasticity. Pathol Res Pract. 1999;195(6):379–89.
111. Ulianich L, Garbi C, Treglia AS, Punzi D, Miele C, Raciti GA, et al. ER stress is associated with dedifferentiation and an epithelial-to-mesenchymal transition-like phenotype in PC Cl3 thyroid cells. J Cell Sci. 2008;121(Pt 4):477–86.
112. Inoki K, Mori H, Wang J, Suzuki T, Hong S, Yoshida S, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. J Clin Invest. 2011;121(6):2181–96.
113. Lawson WE, Cheng DS, Degryse AL, Tanjore H, Polosukhin VV, Xu XC, et al. Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs. Proc Natl Acad Sci U S A. 2011;108(26):10562–7.
114. Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE, et al. Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. J Biol Chem. 2011;286(35):30972–80.
115. Julier C, Nicolino M. Wolcott-Rallison syndrome. Orphanet J Rare Dis. 2010;5:29.
116. Wolcott CD, Rallison ML. Infancy-onset diabetes mellitus and multiple epiphyseal dysplasia. J Pediatr. 1972;80(2):292–7.
117. Delepine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet. 2000;25(4):406–9.
118. Zhang P, McGrath B, Li S, Frank A, Zambito F, Reinert J, et al. The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. Mol Cell Biol. 2002;22(11):3864–74.
119. Harding HP, Zyryanova AF, Ron D. Uncoupling proteostasis and development in vitro with a small molecule inhibitor of the pancreatic endoplasmic reticulum kinase, PERK. J Biol Chem. 2012;287(53):44338–44.
120. Gupta S, McGrath B, Cavener DR. PERK (EIF2AK3) regulates proinsulin trafficking and quality control in the secretory pathway. Diabetes. 2010;59(8):1937–47.
121. Sowers CR, Wang R, Bourne RA, McGrath BC, Hu J, Bevilacqua SC, et al. The protein kinase PERK/EIF2AK3 regulates proinsulin processing not via protein synthesis but by controlling endoplasmic reticulum chaperones. J Biol Chem. 2018;293(14):5134–49.
122. Darlington GJ. Molecular mechanisms of liver development and differentiation. Curr Opin Cell Biol. 1999;11(6):678–82.
123. Zhang W, Feng D, Li Y, Iida K, McGrath B, Cavener DR. PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. Cell Metab. 2006;4(6):491–7.
124. Alam M, Gonzalez R, Delarosa A, Bobek J, Dokainish H, Lakkis N. In vitro inhibition of platelet aggregation in response to increasing concentrations of tirofiban in patients with significant renal insufficiency. Am Heart Hosp J. 2009;7(1):17–20.

125. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF. Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet. 1999;23(1):32–40.
126. Li Y, Iida K, O'Neil J, Zhang P, Li S, Frank A, et al. PERK eIF2alpha kinase regulates neonatal growth by controlling the expression of circulating insulin-like growth factor-I derived from the liver. Endocrinology. 2003;144(8):3505–13.
127. Trivier E, De Cesare D, Jacquot S, Pannetier S, Zackai E, Young I, et al. Mutations in the kinase Rsk-2 associated with Coffin-Lowry syndrome. Nature. 1996;384(6609):567–70.
128. Xing J, Ginty DD, Greenberg ME. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science. 1996;273(5277):959–63.
129. Bruning JC, Gillette JA, Zhao Y, Bjorbaeck C, Kotzka J, Knebel B, et al. Ribosomal subunit kinase-2 is required for growth factor-stimulated transcription of the c-Fos gene. Proc Natl Acad Sci U S A. 2000;97(6):2462–7.
130. Dufresne SD, Bjorbaek C, El-Haschimi K, Zhao Y, Aschenbach WG, Moller DE, et al. Altered extracellular signal-regulated kinase signaling and glycogen metabolism in skeletal muscle from p90 ribosomal S6 kinase 2 knockout mice. Mol Cell Biol. 2001;21(1):81–7.
131. Poteet-Smith CE, Smith JA, Lannigan DA, Freed TA, Sturgill TW. Generation of constitutively active p90 ribosomal S6 kinase in vivo. Implications for the mitogen-activated protein kinase-activated protein kinase family. J Biol Chem. 1999;274(32):22135–8.
132. Ducy P, Karsenty G. Two distinct osteoblast-specific cis-acting elements control expression of a mouse osteocalcin gene. Mol Cell Biol. 1995;15(4):1858–69.
133. Schinke T, Karsenty G. Characterization of Osf1, an osteoblast-specific transcription factor binding to a critical cis-acting element in the mouse Osteocalcin promoters. J Biol Chem. 1999;274(42):30182–9.
134. Dobreva G, Chahrour M, Dautzenberg M, Chirivella L, Kanzler B, Farinas I, et al. SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. Cell. 2006;125(5):971–86.
135. Wang W, Lian N, Ma Y, Li L, Gallant RC, Elefteriou F, et al. Chondrocytic Atf4 regulates osteoblast differentiation and function via Ihh. Development. 2012;139(3):601–11.
136. Saito A, Ochiai K, Kondo S, Tsumagari K, Murakami T, Cavener DR, et al. Endoplasmic reticulum stress response mediated by the PERK-eIF2(alpha)-ATF4 pathway is involved in osteoblast differentiation induced by BMP2. J Biol Chem. 2011;286(6):4809–18.
137. Malzer E, Dominicus CS, Chambers JE, Dickens JA, Mookerjee S, Marciniak SJ. The integrated stress response regulates BMP signalling through effects on translation. BMC Biol. 2018;16(1):34.
138. Kang K, Ryoo HD, Park JE, Yoon JH, Kang MJ. A Drosophila reporter for the translational activation of ATF4 marks stressed cells during development. PLoS One. 2015;10(5):e0126795.
139. Hewes RS, Schaefer AM, Taghert PH. The cryptocephal gene (ATF4) encodes multiple basic-leucine zipper proteins controlling molting and metamorphosis in Drosophila. Genetics. 2000;155(4):1711–23.
140. Kang MJ, Vasudevan D, Kang K, Kim K, Park JE, Zhang N, et al. 4E-BP is a target of the GCN2-ATF4 pathway during Drosophila development and aging. J Cell Biol. 2017;216(1):115–29.
141. Eyries M, Montani D, Girerd B, Perret C, Leroy A, Lonjou C, et al. EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. Nat Genet. 2014;46(1):65–9.
142. Best DH, Sumner KL, Austin ED, Chung WK, Brown LM, Borczuk AC, et al. EIF2AK4 mutations in pulmonary capillary hemangiomatosis. Chest. 2014;145(2):231–6.
143. International PPHC, Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA III, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nat Genet. 2000;26(1):81–4.
144. Montani D, Achouh L, Dorfmuller P, Le Pavec J, Sztrymf B, Tcherakian C, et al. Pulmonary veno-occlusive disease: clinical, functional, radiologic, and hemodynamic characteristics and outcome of 24 cases confirmed by histology. Medicine. 2008;87(4):220–33.

145. Graf S, Haimel M, Bleda M, Hadinnapola C, Southgate L, Li W, et al. Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. Nat Commun. 2018;9(1):1416.
146. Cao Z, Umek RM, McKnight SL. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. Genes Dev. 1991;5(9):1538–52.
147. Yeh WC, Cao Z, Classon M, McKnight SL. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. Genes Dev. 1995;9(2):168–81.
148. Wu Z, Xie Y, Bucher NL, Farmer SR. Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. Genes Dev. 1995;9(19):2350–63.
149. Christy RJ, Kaestner KH, Geiman DE, Lane MD. CCAAT/enhancer binding protein gene promoter: binding of nuclear factors during differentiation of 3T3-L1 preadipocytes. Proc Natl Acad Sci U S A. 1991;88(6):2593–7.
150. Altiok S, Xu M, Spiegelman BM. PPARgamma induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. Genes Dev. 1997;11(15):1987–98.
151. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev. 2002;16(1):22–6.
152. Ron D, Habener JF. CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. Genes Dev. 1992;6(3):439–53.
153. Batchvarova N, Wang XZ, Ron D. Inhibition of adipogenesis by the stress-induced protein CHOP (Gadd153). EMBO J. 1995;14(19):4654–61.
154. Tang QQ, Lane MD. Role of C/EBP homologous protein (CHOP-10) in the programmed activation of CCAAT/enhancer-binding protein-beta during adipogenesis. Proc Natl Acad Sci U S A. 2000;97(23):12446–50.
155. Darlington GJ, Ross SE, MacDougald OA. The role of C/EBP genes in adipocyte differentiation. J Biol Chem. 1998;273(46):30057–60.
156. Basseri S, Lhotak S, Sharma AM, Austin RC. The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response. J Lipid Res. 2009;50(12):2486–501.
157. Ishikawa T, Okada T, Ishikawa-Fujiwara T, Todo T, Kamei Y, Shigenobu S, et al. ATF6alpha/beta-mediated adjustment of ER chaperone levels is essential for development of the notochord in medaka fish. Mol Biol Cell. 2013;24(9):1387–95.
158. Walsh K. Coordinate regulation of cell cycle and apoptosis during myogenesis. Prog Cell Cycle Res. 1997;3:53–8.
159. Fernando P, Kelly JF, Balazsi K, Slack RS, Megeney LA. Caspase 3 activity is required for skeletal muscle differentiation. Proc Natl Acad Sci U S A. 2002;99(17):11025–30.
160. Nakanishi K, Sudo T, Morishima N. Endoplasmic reticulum stress signaling transmitted by ATF6 mediates apoptosis during muscle development. J Cell Biol. 2005;169(4):555–60.
161. Fischer H, Koenig U, Eckhart L, Tschachler E. Human caspase 12 has acquired deleterious mutations. Biochem Biophys Res Commun. 2002;293(2):722–6.
162. Okada T, Haze K, Nadanaka S, Yoshida H, Seidah NG, Hirano Y, et al. A serine protease inhibitor prevents endoplasmic reticulum stress-induced cleavage but not transport of the membrane-bound transcription factor ATF6. J Biol Chem. 2003;278(33):31024–32.
163. Nakanishi K, Dohmae N, Morishima N. Endoplasmic reticulum stress increases myofiber formation in vitro. FASEB J. 2007;21(11):2994–3003.
164. Wu J, Ruas JL, Estall JL, Rasbach KA, Choi JH, Ye L, et al. The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex. Cell Metab. 2011;13(2):160–9.
165. Jang WG, Kim EJ, Kim DK, Ryoo HM, Lee KB, Kim SH, et al. BMP2 protein regulates osteocalcin expression via Runx2-mediated Atf6 gene transcription. J Biol Chem. 2012;287(2):905–15.

166. Kim JW, Choi H, Jeong BC, Oh SH, Hur SW, Lee BN, et al. Transcriptional factor ATF6 is involved in odontoblastic differentiation. J Dent Res. 2014;93(5):483–9.
167. Xu M, Gelowani V, Eblimit A, Wang F, Young MP, Sawyer BL, et al. ATF6 is mutated in early onset photoreceptor degeneration with macular involvement. Invest Ophthalmol Vis Sci. 2015;56(6):3889–95.
168. Kroeger H, Grimsey N, Paxman R, Chiang WC, Plate L, Jones Y, et al. The unfolded protein response regulator ATF6 promotes mesodermal differentiation. Sci Signal. 2018;11(517). https://doi.org/10.1126/scisignal.aan5785; pii: eaan5785.
169. Honma Y, Kanazawa K, Mori T, Tanno Y, Tojo M, Kiyosawa H, et al. Identification of a novel gene, OASIS, which encodes for a putative CREB/ATF family transcription factor in the long-term cultured astrocytes and gliotic tissue. Brain Res Mol Brain Res. 1999;69(1):93–103.
170. Kondo S, Murakami T, Tatsumi K, Ogata M, Kanemoto S, Otori K, et al. OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. Nat Cell Biol. 2005;7(2):186–94.
171. Murakami T, Kondo S, Ogata M, Kanemoto S, Saito A, Wanaka A, et al. Cleavage of the membrane-bound transcription factor OASIS in response to endoplasmic reticulum stress. J Neurochem. 2006;96(4):1090–100.
172. Hosoya T, Takizawa K, Nitta K, Hotta Y. Glial cells missing: a binary switch between neuronal and glial determination in Drosophila. Cell. 1995;82(6):1025–36.

Chapter 3
The Regulation of the Unfolded Protein Response and Its Roles in Tumorigenesis and Cancer Therapy

Jordan Morreall, Feng Hong, and Zihai Li

Abstract The unfolded protein response (UPR) of the endoplasmic reticulum (ER) is a highly conserved system by which cells regulate multiple pathways during misfolded protein accumulation. Acute UPR signaling inhibits translation, induces chaperone expression, and activates proteolysis, whereas chronic UPR signaling can lead to apoptosis. Each of the canonical functions of UPR serves as a mechanism that can limit or facilitate tumorigenesis. Tumor cells are able to coopt UPR signaling to facilitate proliferation, transformation, and epithelial-to-mesenchymal transition (EMT) under hypoxia and glucose starvation, potentially causing metastasis. UPR signaling is typically initiated by Glucose-Regulated Protein 78 (GRP78/BiP) binding to unfolded proteins, causing GRP-78 to dissociate from each of the three primary UPR sensors on the ER membrane: protein kinase R-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring protein 1α (IRE1α). Recent studies highlight the complexity of the signaling interactions involved, but also potential clinical opportunities to target unique molecular interfaces. This review discusses the current understanding of UPR pathways, ongoing clinical approaches to manipulate UPR signaling, and future avenues by which cancer therapy may be advanced by utilizing approaches that target the molecules involved in UPR signaling.

Keywords UPR · ER stress · PERK · ATF6α · IRE1α · BiP · GRP78 · Proteostasis · Redox

J. Morreall · F. Hong · Z. Li (✉)
Department of Microbiology and Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA
e-mail: morreall@musc.edu; hongf@musc.edu; zihai@musc.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_3

The Roles of the Unfolded Protein Response

The endoplasmic reticulum (ER) is the organelle primarily responsible for protein processing, folding, and transport. In order to carry out protein folding, the ER must maintain an internal environment in which disulfide bonds can form. To do so, ER function relies upon a high calcium concentration, oxidizing conditions, and a variety of chaperone proteins and protein folding enzymes [1, 2]. A variety of extrinsic and intrinsic conditions can inhibit ER function, and thus cause ER stress. ER stress, due to the accumulation of misfolded proteins in the ER, activates a network of pathways termed the unfolded protein response (UPR). Acute UPR activation facilitates the alleviation of the causative ER stress through upregulation of molecular chaperones, downregulation of translation machinery, and induction of the ER-associated degradation (ERAD) system by which misfolded proteins are removed from the ER. However, continued accumulation of unfolded proteins can lead to apoptosis via chronic UPR activation [3].

The first evidence of a coordinated UPR arose from studies illustrating transcriptional upregulation in response to glucose starvation [4]. More evidence came from the discovery that one such glucose-regulated protein was identical to Binding Immunoglobulin Protein (BiP/GRP78/HSPA5), known to bind unfolded proteins [5]. The UPR was first experimentally validated by the demonstration that misfolded hemagglutinin alone could induce the expression of the known ER stress response proteins BiP and 94-kDa Glucose-Regulated Protein (GRP94) [6]. BiP was identified as a protein bound to unsecreted immunoglobulin heavy chains [7], suggestive of its role later identified as a molecular chaperone [8]. Under non-stress conditions, BiP is bound to three ER membrane proteins: PRKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring protein 1α (IRE1α). Unfolded proteins in the ER bind free BiP and decrease the steady-state levels of this chaperone, causing it to be released from these sensors, after which they undergo activation and initiation of further signaling cascades [9].

Interestingly, UPR signaling can be initiated by signals independent of ER stress, including Vascular Endothelial Growth Factor (VEGF) stimulation [10]. Likewise, although often the target of PERK, the downstream UPR signaling molecule eukaryotic translation initiation factor 2α (eIF2α) can be phosphorylated by other kinases such as protein kinase R (PKR), activated by binding dsRNA [11]; general control nonderepressible 2 (GCN2) kinase, activated by amino acid depletion [12]; and heme-regulated eIF2α (HRI) kinase, activated by diminished heme levels, typically leading to apoptosis [13]. UPR signaling can also be induced by estrogen signaling through estrogen receptor α (ERα), causing the transient anti-apoptotic opening of ER calcium channels and the upregulation of GRP78/BiP [14]. Nonetheless, UPR signaling is primarily mediated by PERK, Activating Transcription Factor 4 (ATF4), and IRE1α, through which this chapter will describe the functions of the UPR in cancer cells and ways in which its components can be targeted.

Regulation of the Unfolded Protein Response in Cancer Cells

UPR signaling provides several avenues through which cells are able to avoid tumorigenesis. Tumor cells are characterized by a high metabolic rate than can impose ER stress via the rampant production of proteins. However, acute UPR signaling can ameliorate such ER stress, while chronic UPR signaling typically leads to apoptosis, or possibly even H-ras-induced senescence [15]. Nevertheless, some cells undergo oncogenic transformation in a manner that is facilitated by UPR. For example, Myc can stimulate PERK signaling that causes increased protective autophagy and thus cell survival and tumor formation [16].

Tumor cells, particularly those within solid tumors, often proliferate faster than the vascular systems that would provide them with normal cellular oxygenation and glucose supply. Glucose starvation or hypoxia each contributes to a diminished redox potential that limits the formation of disulfide bonds, contributing to an inherent ER stress on hypoxic cells that causes UPR activation [17, 18]. Excess glucose or dietary lipids can also cause an increase in ER stress [19]. The combination of ER stress and glucose starvation induces autophagy, in which cellular components are engulfed and digested, potentially facilitating either cell death or survival [20]. Autophagy is mediated by eIF2α phosphorylation [21].

The high metabolic demands of tumor cell proliferation necessitate increased angiogenesis, which can be mediated through UPR signaling. In addition to hypoxia-stimulated HIF-1, angiogenesis has also been shown to depend on PERK phosphorylation of eIF2α [17]. Moreover, PERK contributes to transcriptional regulation that stimulates angiogenesis within the tumor microenvironment, upregulating transcripts for cellular adhesion protein VCIP, integrins, and factors promoting capillary remodeling [22]. PERK and ATF4 also stimulate the angiogenic factors VEGF, Fibroblast Growth Factor 2 (FGF-2), and Interleukin 6 (IL-6), while inhibiting anti-angiogenic factors such as Thrombospondin 1 (THBS1), Chemokine Ligand 14 (CXCL14), and Chemokine Ligand 10 (CXCL10), as studied under glucose starvation-induced ER stress [23]. Additionally, hypoxia and glucose starvation can stimulate VEGF-A upregulation through IRE1, which substantially affects tumor angiogenesis and proliferation [24]. Blocking IRE1α signaling not only attenuates VEGF-A signaling but also the proangiogenic factors Interleukin 1β (IL-1β), IL-6, and Interleukin 8 (IL-8) [25]. Moreover, IRE1α signaling is involved in the expression of anti-angiogenic molecules such as SPARC, thrombospondin 1, and decorin. All of these molecules are expressed in the matrix and promote mesenchymal differentiation and, paradoxically, the invasiveness of gliomas [25].

IRE1α can play a major role in regulating migration of glioma cells by downregulating stress fibers and RhoA activity, ultimately governing the secretome of cancer cells [26]. Other models of ischemia have illustrated a role for IRE1α inhibiting angiogenesis due to degradation of the transcript for the angiogenic signal netrin-1 via regulated IRE1-dependent decay (RIDD) [27]. PERK and IRE1α, in particular, mediate ER stress signaling that regulates the tumor microenvironment via angiogenesis.

Moreover, breast and lung cancer carcinomas can undergo higher levels of GRP78/BiP expression, poorer differentiation, and a more mesenchymal phenotype. Interestingly, such cells with a more mesenchymal phenotype have a proliferative advantage under complete glucose starvation [28]. Such evidence suggests that the UPR may facilitate the epithelial-mesenchymal transition (EMT) in cancer cells; the EMT is a driver of a metastatic phenotype that is a mechanism of metastasis [29]. Moreover, the ER stress endemic in many tumor cells is not necessarily restricted to the tumor itself. Tumor cells under ER stress may secrete soluble factors that induce ER stress in macrophages, thus potentially stimulating a proinflammatory cellular response within the tumor microenvironment [30]. Furthermore, UPR signaling from tumor cells causes myeloid cells in the tumor microenvironment to become polarized, which limits T cell activation and expansion [31] (Fig. 3.1).

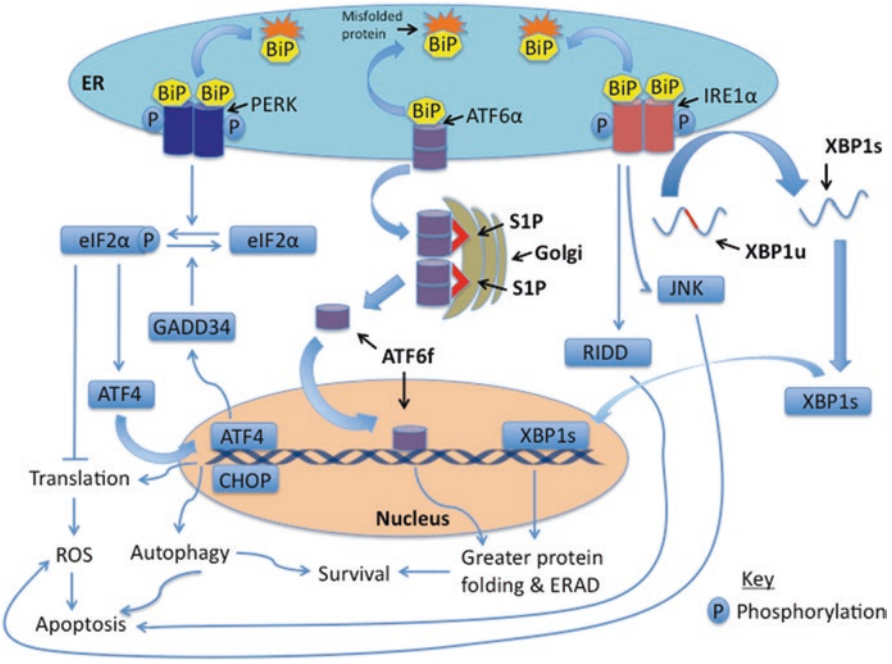

Fig. 3.1 UPR signaling regulates survival and apoptosis. Misfolded proteins are bound by the chaperone GRP78/BiP, which causes its dissociation from PERK, ATF6α, and IRE1α. PERK is then able to undergo dimerization and autophosphorylation, which allows it to phosphorylate eIF2α. Phospho-eIF2α is then able to promote ATF4 signaling via transcriptional regulation with CHOP that facilitates autophagy. Meanwhile, activated ATF6α translocates to the Golgi apparatus, where the S1P and S2P proteases cleave the protein into the active form ATF6f. ATF6f then acts as a transcription factor in the nucleus, where it enhances expression of chaperones and proteins involved in Endoplasmic Reticulum-Associated Degradation (ERAD). IRE1α is also able to undergo dimerization and phosphorylation, allowing it to promote the transcriptional processing of XBP1 transcripts (XBP1u) into the active form XBP1s, which after translation acts as a transcription factor for chaperones and ERAD proteins. ERAD serves as a pro-survival mechanism, while the RIDD and JNK pathways stimulated by IRE1α facilitate apoptotic signaling

PERK Signaling

PERK is a transmembrane protein that contains a serine/threonine kinase domain on its cytosolic face. The cytosolic portion of PERK is bound by heat shock protein 90 (HSP90) in the absence of ER stress, while the portion within the ER lumen is bound by GRP78/BiP. Under ER stress, GRP78/BiP binds unfolded proteins, dissociating from PERK and leaving it free to undergo activation via homodimerization and autophosphorylation [32]. One recent study shows that CNPY2, a ER protein, is dissociated from GRP78/BiP under ER stress, and then binds and activates PERK. [33]. PERK is then able to phosphorylate eIF2α S51, which limits the availability of eukaryotic initiation factor 2 (EIF2)-guanosine triphosphate (GTP)-$tRNA_{met}$ and thus the initiation of translation [11]. This phosphorylation allows tight binding of eIF2α to guanosine diphosphate (GDP), which prevents eIF2B from undergoing GTP binding and exchange and further blocking protein synthesis [34]. The activation of PERK occurs after that of ATF6α and IRE1α [35, 36].

Another consequence of PERK-eIF2α activation is the induction of translation of certain mRNAs, such as ATF4 and proteins that transport amino acids [37]. ATF4 is then able to serve as a transcription factor by upregulating genes important in antioxidant defenses as well as amino acid production [38]. Other targets of ATF4 include growth arrest and DNA damage inducible protein 34 (GADD34), leading to eIF2α dephosphorylation, and CCAAT/enhancer binding protein homologous protein (CHOP) [39, 40]. Induction of CHOP is a major mechanism by which ER stress induces apoptosis [41]. Shortly after the induction of ER stress, PERK also induces microRNA 211 (miR-211), which causes histone methylation that limits CHOP expression, a mechanism by which acute ER stress does not cause the apoptosis seen under chronic ER stress [42].

On the other hand, chronic PERK-eIF2α phosphorylation can lead to apoptosis via CHOP signaling during the diminution of IRE1α and ATF6α signaling, causing decreased tumorgenic potential [11, 35]. CHOP can cause apoptosis by inducing B cell lymphoma 2-interacting mediator of cell death (BIM) while facilitating B cell lymphoma-2 associated X protein (BAX) shuttling to the mitochondria [43, 44]. However, during glucose starvation or hypoxia, diminished PERK levels can lead to decreased tumor cell survival and diminished metabolic ATP production, partly due to limited activation of AKT [45]. Likewise, PERK appears to be an important mediator of EMT by signaling through its downstream effector LAMP3, expression of both of which is critical for metastasis under hypoxia [40]. Interestingly, PERK is also necessary for the regulatory ubiquitination of 40S ribosomal subunits, without which cell survival is diminished during chronic UPR signaling [46].

PERK seems to play an important role in *Neu*-dependent mammary tumor formation and metastasis. However, inactivating PERK increases the frequency of genomic abnormalities, underpinning an increase in spontaneous mammary tumor formation [47]. Activation of PERK increases the frequency of oncogenic transformation induced by MYC via autophagy [16]. Regulation of CHOP by PERK is a critical mechanism of stemming tumorigenesis, as demonstrated in mouse models of lung cancer and hepatocellular carcinoma [48, 49].

ATF6 Signaling

GRP78/BiP binding to unfolded proteins also causes its dissociation from ATF6, allowing the transmembrane protein to shuttle from the ER to the Golgi apparatus. In the Golgi, ATF6 is cleaved into the active transcription factor ATF6f by the proteases site-1 protease (S1P) and site-2 protease (S2P) [50]. Active ATF6f is then able to migrate to the nucleus, where it stimulates the expression of chaperones, X-box binding protein 1 (*Xbp1*), and proteins important in ERAD. An alternative isoform, ATF6β, represses the transcription factor activity of ATF6α [51].

As a transcription factor, ATF6f serves as an important regulator of GRP78/BiP. ER stress causes ATF6f to quickly induce expression of GRP78/BiP, allowing the chaperone to accumulate and dampen UPR signaling while binding unfolded proteins. However, GRP78/BiP is overexpressed in a variety of cancers, and even can be found on the cell surface, causing aberrant signaling [52]. The role of ATF6f regulating GRP78/BiP expression may contribute to its role in promoting hepatocarcinogenesis [53]. Susceptibility to hepatocellular carcinoma is increased in patients carrying a point mutation in *ATF6* that increases ATF6 expression and transcription factor activity [54]. The degree of GRP78/BiP overexpression is correlated with the invasiveness of cancer cells, though also with their responsiveness to therapeutic intervention [55]. Presumably, overexpression of GRP78/BiP allows some cancer cells to maintain high levels of ER stress without the growth-limiting effects of UPR signaling.

IRE1α Signaling

IRE1α is a transmembrane protein that contains a cytosolic serine/threonine kinase domain. Without ER stress, HSP90 (as well as HSP72) binds the cytosolic face of IRE1α, while GRP78/BiP binds to its luminal side [56, 57]. When released from GRP78/BiP, IRE1α undergoes oligomerization and activation of both its endoribonuclease and kinase activities, allowing IRE1α to undergo autophosphorylation [32]. IRE1α is then able to cleave unspliced *Xbp1u* mRNA, removing an intronic sequence that creates a transcript with a frameshift called *Xbp1s* [58, 59]. *Xbp1s* can then be translated into a protein that regulates a number of chaperone and ERAD genes. Interestingly, overexpression of XBP1s inhibits CHOP and thus provides a pro-survival signal [60]. One upstream regulator of XBP1 has been identified in *C. elegans*, a conserved ATPase called RUVB-2, that represses ER stress response via XBP1, and must undergo degradation by the ATPase cell division protein 47 (CDC-48) in order to allow UPR [61].

Although IRE1α signaling facilitates cell survival during acute ER stress, chronic UPR signaling causes diminished IRE1α activation that may lead to apoptosis [62]. One mechanism for the loss of IRE1α activity may be the binding of *Xbp1u* to XBP1s and ATF6α that facilitates their degradation [63]. Nevertheless, apoptosis can arise from chronic IRE1α stimulation as well. IRE1α represses translation by

cleaving transcripts via RIDD, including Ire1α mRNA and Xbp1 mRNA [64, 65]. RIDD also targets microRNAs (miRNAs) such as miR-17, miR34a, miR-96, and miR-125b, and thereby derepresses caspase 2 [66]. Another avenue through which IRE1α could induce apoptosis is through binding tumor necrosis factor receptor-associated factor 2 (TRAF2), recruiting apoptosis signal-regulating kinase 1 (ASK1) and JUN N-terminal kinase (JNK), which activates BIM while inactivating B cell lymphoma 2 (BCL-2) [67].

IRE1α plays a critical role through XBP1 signaling in stimulating the differentiation of mature B cells into plasma cells, as well as the maintenance of B cell immunity [68, 69]. High XBP1s expression is associated with the development of multiple myeloma [70]. In human multiple myeloma cell lines, loss-of-function mutations in IRE1α or XBP1s increase resistance to proteasome inhibitors, a standard treatment [71]. *IRE1A* loss-of-function mutations have also been found in a variety of other cancers, while XBP1 is known to suppress gut tumorigenesis in mice [72, 73]. Conversely, high expression of XBP1 is implicated in triple-negative breast cancer, suggesting that XBP1 may play a role in promoting stem-like phenotypes [74]. Furthermore, diminished levels of XBP1 have been observed to prevent the differentiation of myeloma cells, characteristic of tumor cells, whereas the activation of the intact UPR stimulates myeloma cell differentiation [75].

Coordination of Signaling from PERK, ATF6, and IRE1α

The central coordination of UPR signaling lies in the upstream regulator GRP78/BiP. Cancer cells are able to diminish apoptotic signaling that arises from UPR activation through overexpression of GRP78/BiP [76]. However, recent studies have identified other shared UPR regulators. cAMP response element binding protein (CREB) regulates PERK and IRE1α by binding their promoters and controlling their expression. CREB has been identified as an important contributor to both UPR-mediated lung metastasis and ER stress-induced cell death [77]. Similarly, transmembrane protein 33 (TMEM33) is upregulated in response to ER stress, binds to PERK, increases pro-apoptotic caspase signaling, and activates IRE1α and eIF2α [78].

Chronic UPR in normal cells causes dwindling signals from IRE1α and ATF4, allowing CHOP induction from PERK signaling to cause apoptosis [35]. Some cancer cells evade apoptosis through constitutively active IRE1α signaling [79]. The upregulation of CHOP induces the AKT antagonist TRIB3 and thereby blocks proliferative mTOR signaling to cause autophagy [80]. The translation inhibition caused by eIF2α phosphorylation limits cyclin D1 availability, causing G1 arrest [81]. In this way, UPR activation in cancer cells may promote a quiescent phenotype allowing survival under stress conditions. On the other hand, some cancer patients have been identified in whom RIDD deficiency permitted tumor cell survival by escaping apoptosis [82].

The activation of some branches of the UPR can also stimulate its other branches. For example, the activation of PERK and eIF2α leads to increased levels and trafficking of ATF6α [83]. Androgen signaling can also simultaneously affect several branches of UPR signaling. One study identified androgen receptor signaling as activating IRE1α in a pro-growth manner while inhibiting PERK in prostate cancer cells, as well as a correlation between androgen receptor and UPR gene expression [84]. The modulation of multiple UPR branches can also occur from signaling by cell cycle regulators such as cyclin D1, which promotes ER stress-induced apoptosis [85]. ER stress-induced apoptosis involves decreasing levels of apoptosis-inducing E2F1, mediated by ATF6 and IRE1, during the later stages of ER stress response. The knockdown of E2F1 causes increased ER stress-induced apoptosis [86].

Pharmacological Interventions in UPR Biology

The UPR involves complex signaling that has been implicated in a variety of pathologies. However, since the activation of the UPR can have both pro-survival and pro-apoptotic effects, there is considerable complexity in the pharmacological intervention in cancer UPR signaling. ER stress is found at higher levels within many tumor cells, especially in cells with a secretory phenotype such as multiple myeloma. Such cells rely on a continuous induction of cellular proliferation and signaling that causes ER stress. Many tumor cells can therefore be targeted by imposing ER stress, which can cause cells already under ER stress to undergo apoptosis. For example, bortezomib is a 26S proteasome inhibitor that is used widely to treat multiple myeloma and mantle cell lymphoma. Bortezomib induces the expression of CHOP, PERK, and ATF4 in multiple myeloma cells [87]. IRE1α and XBP1 are both necessary in order for cells to be sensitive to such proteasome inhibitors since tumor preplasmablasts rely on these proteins in order to undergo maturation into immunoglobulin-secreting B cells [71]. On the other hand, the reliance of tumor cells on ER function can leave them vulnerable to inhibition of ER components (Table 3.1).

PERK Signaling

PERK signaling can be stimulated by a range of insults, exemplified by clinical techniques to upregulate PERK that now can involve non-pharmacological means. For instance, while conventional radiation has considerable systemic dose-limiting toxicity, heavy ion radiation is able to induce localized cytotoxic autophagy with great efficiency, an effect which is partly mediated by stimulating the UPR via the PERK axis while inhibiting Akt-mTOR [127]. This technique is limited by the availability of heavy ion radiation, although it has shown greater efficacy than traditional radiotherapy. Another therapeutic agent that can induce apoptosis by

Table 3.1 Pharmacological interventions in UPR signaling

Target	Drugs	Secondary targets and references	Cancer clinical trials
UPR induction	Sorafenib	Induces cytotoxic VCP phosphorylation [88]	FDA approved for renal carcinoma and hepatocellular carcinoma
GRP78/BiP expression	Versipelostatin	Inhibits induction of GRP78/BiP and UPR signaling in glucose-starved cells [89]	Preclinical
	PAT-SM6	Binds to BiP on cancer cell surface [90]	Phase 1/2 in multiple myeloma
	DHA	• Blocks surface GRP78 expression • Inhibits PERK [91]	Phase 2/3 in solid tumors
	Arctigenin	• Blocks the induction of BiP and GRP94 during glucose starvation • Prevents AKT activation during glucose starvation [92]	Preclinical
	EGCG (epigallocatechin gallate)	Targets GRP78/BiP ATP-binding domain [93]	Phase 1/2
	Nelfinavir	• Inhibits HSP90 • Inhibits S2P and thereby induces ATF6 • Activates caspases 3, 7, and 8 • Inhibits AKT, causing diminished VEGFA and HIF1α [94]	Phase 1/2 in solid tumors and multiple myeloma
Proteasome	Carfilzomib	• Promotes NF-κB activation • Induces pro-apoptotic BCL2-Interacting Killer (BIK) and anti-apoptotic Myeloid Cell Leukemia 1 (MCL1) [95]	FDA approved for multiple myeloma; Phase 1/2 in hematopoietic malignancies and lung cancer
	MLN9708	• Activates caspases 3, 8, and 9 • Upregulates p53, p21, NOXA, p53-Upregulated Modulator of Apoptosis (PUMA), and E2F • Inhibits NF-κB [96]	Phase 1 in solid tumors; Phase 1/2 in hematopoietic malignancies; Phase 3 in multiple myeloma
	Marizomib	Upregulates caspase 8 and ROS-mediated apoptosis [97]	Phase 1 in solid tumors and hematopoietic malignancies; Phase 1/2 in multiple myeloma
	Falcarindiol	Inhibits proteasome [98]	Preclinical

(continued)

Table 3.1 (continued)

Target	Drugs	Secondary targets and references	Cancer clinical trials
	NPI-0052	Blocks NF-κB activity [99]	Phase 1 in solid tumors and hematopoietic malignancies; Phase 1/2 in multiple myeloma
	Bortezomib	• Inhibits IRE1α–XBP1 and NF-κB pathways • Induces expression of NOXA • Triggers immunogenic cell death [100]	FDA approved for multiple myeloma and mantle cell lymphoma; Phase 1/2 in solid tumors
	MG-132	Cytotoxic activation of UPR [101]	Preclinical
PERK and eIF2α phosphorylation	GSK2606414	Inhibits active site of PERK [102]	Preclinical
	6-shogaol	• Promotes light chain 3 (LC3) cleavage • Induces cell death through autophagy [103]	Preclinical
	GSK2656157	• Binds PERK ATP-binding site [104]	Preclinical
HSP90	AT13387	• Promotes senescence • Represses epidermal growth factor receptor (EGFR), AKT, and Cyclin-Dependent Kinase 4 (CDK4) • Induces p27 [105]	Phase 1/2 in solid tumors
	17-AAG	Apoptotic UPR activation [106]	Phase 2/3
	Tanespimycin	• Blocks 20S proteasome chymotrypsis • Limits cell proliferation via BRAF expression • Interferes with VEGFA and causes apoptosis [107]	Phase 1/2 in solid tumors and hematopoietic malignancies; Phase 3 in multiple myeloma
	SNX-5422	NA [108]	Phase 1 in solid tumors and hematopoietic malignancies; Phase 2 in TP53-null tumors
	Ganetespib	• Inhibits AKT • Represses HIF1α and signal transducer and activator of transcription 3 (STAT3) [109]	Phase 1/2 in solid tumors and hematopoietic malignancies; Phase 3 in non-small-cell lung cancer, Acute Myeloid Leukemia, andMyelodysplastic Syndrome

(continued)

Table 3.1 (continued)

Target	Drugs	Secondary targets and references	Cancer clinical trials
	AUY922	• Suppresses AKT and ERK only in Phosphatase and Tensin homolog (*PTEN*)-null esophageal squamous cancer cells • Inhibits NF-κB • Promotes apoptosis by repressing Rapidly Accelerated Fibrosarcoma 1 (RAF1) [110]	Phase 1/2 in solid tumors and hematopoietic malignancies
	PU-H71	• Represses AKT, ERK, RAF1, MYC, KIT, Insulin-Like Growth Factor 1 Receptor (IGF1R), Telomerase Reverse Transcriptase (TERT) and Ewing sarcoma-Friend Leukemia Integration 1 (EWS–FLI1) in Ewing sarcoma cells • Facilitates degradation of Inhibitor of nuclear factor Kappa-β Kinase subunit β (IKKβ) and activated AKT and B Cell Lymphoma Extra large (BCL-X) [111]	Phase 1 in solid tumors and hematopoietic malignancies
	Debio 0932	NA [112]	Phase 1 in solid tumors and hematopoietic malignancies
	XL888	• Facilitates degradation of CDK4 and WEE1 • Inhibits AKT signaling • Increases BIM expression and decreases MCL1 expression [113]	Phase 1 in melanoma
	IPI-504	• Binds to ATP-binding site on HSP90 • Disrupts transcription factor activity of XBP1 and ATF6 • Interferes with PERK phosphorylation of eIF2α • Limits BiP buildup [114]	Phase 1/2 in solid tumors and hematopoietic malignancies; Phase 3 in gastrointestinal stromal tumors
Pan-deacetylase	Panobinostat	• Induces BiP, ATF4, and CHOP, IRE1α and eIF2α activation • Induces BIK, BIM, BAX, Bcl-2 Antagonist/Killer (BAK), and caspase 7 activity [115]	FDA approved for multiple myeloma; Phase 1/2 in solid tumors and hematopoietic malignancies; Phase 3 in hematopoietic malignancies

(continued)

Table 3.1 (continued)

Target	Drugs	Secondary targets and references	Cancer clinical trials
WNT signaling	Pyrvinium	• Represses BIP and GRP94 induction during glucose starvation [116]	FDA-approved anthelmintic agent; preclinical for cancer
Anti-diabetic biguanides	Metformin	• Represses XBP1 and ATF4 under glucose starvation [117]	FDA-approved anti-diabetes drug; Phase 1/2 in solid tumors and hematopoietic malignancies; Phase 3 in solid tumors
IRE1α	Sunitinib	Pro-proliferative kinases [118]	Phase II for multiple myeloma; FDA approved for renal cell carcinoma
	STF-083010	• Limits endonuclease activity of IRE1 endonuclease [119]	Preclinical
	MKC-3946	• Impedes IRE1α endonuclease domain • Increases apoptosis when coadministered with bortezomib and 17-AAG [120]	Preclinical
	Toyocamycin	• Cytotoxic inhibition of *XBP1* mRNA splicing [121]	Preclinical
	4μ8C	Inhibition of *XBP1* mRNA splicing [122]	Preclinical for multiple myeloma
	MKC-3946	• Inhibition of *XBP1* mRNA splicing • Sensitization to bortezomib [123]	Preclinical for multiple myeloma
VCP	DBeQ	• Buildup of ubiquitinated proteins and LC3-II [124]	Preclinical
	ML240	• Buildup of ubiquitinated proteins and LC3-II [125]	Preclinical
	Eeyarestatin	• Induction of UPR genes • Buildup of ubiquitinated proteins • Increased bortezomib sensitivity • Inhibits tumor growth [126]	Preclinical

Major avenues of pharmacological interventions in cancer include proteasome inhibition, inhibition of the UPR regulators HSP90 and GRP78/BiP, and inhibitors of the downstream signaling molecules PERK and IRE1

stimulating PERK signaling is farnesol, which caused cytotoxicity in a leukemia model [128]. Although farnesol is appealing in its natural availability, it has so far been shown to have limited efficacy.

Several PERK modulators have been developed with promising results. For example, GSK2656157 has high specificity for PERK inhibition and targets tumor vasculature, though human use would be limited by pancreatic dysfunction [129]. Future therapeutics may be able to target upstream activators of PERK such as the ER-resident thiol oxidoreductase ERp57, which catalyzes the formation of disulfide bonds, the knockdown of which causes cell death via PERK activation [130]. Likewise, the triterpenoid compound celastrol induces apoptosis in a PERK-dependent manner [131]. Another class of compounds, sulfonamidebenzamides, has been identified as selectively upregulating the CHOP pathway downstream of PERK and inhibiting proliferation in a number of cancer cell lines [132]. Induction of the UPR also has considerable therapeutic value, demonstrated by the efficacy of borrelidin, a threonyl-tRNA synthetase (ThRS) inhibitor that increases XBP1 splicing and causes increased eIF2α activation in an oral squamous cell carcinoma model, in which PERK expression conferred sensitivity to borrelidin [133].

UPR induction can also be mediated by the induction of both metabolic and ER stress, such as by administration of the inhibitory glucose analog 2-deoxy-D-glucose (2DG) that also inhibits N-glycosylation. 2DG has been utilized in studies of Acute Lymphocytic Leukemia (ALL), in which treatment leads to apoptosis via UPR signaling, particularly among BCR-ABL+ ALL cells [134]. Given the limited types of cells sensitive to 2DG, it remains to be seen whether such targeted therapy may hold clinical promise.

ATF6 Signaling

ATF6 signaling is currently an underdeveloped avenue of pharmacological intervention in cancer. Nevertheless, nelfinavir inhibits its downstream target S2P and causes accumulation of ATF6 by preventing its conversion to the active cleavage product ATF6f [135]. Because of the high growth rate of tumor cells, the deprivation of a single amino acid can dramatically reduce their growth potential while posing minimal risk of toxicity for the patient. One such methodology is arginine starvation, which induces chronic ER stress via IRE1α and ATF6 in cancer cell lines. However, arginine starvation alone appears to be relatively cytostatic, so in order to induce toxicity, studies have supplemented this therapy with the arginine analog canavinine, which may enhance its efficacy [136].

IRE1α Signaling

Estrogen receptor signaling is especially important in breast cancer, in which resistance to chemotherapeutics and UPR activation are associated in triple-negative breast cancers. Estrogen receptor β 1 (ERβ1) induces the degradation of IRE1α, underlying the association between IRE1α levels and activity and the survival of ERβ1 positive cells. While ERβ1 promotes ER stress-induced apoptosis, estrogen receptor α (ERα) regulates XBP1 expression. These findings illustrate an opportunity to regulate UPR-associated breast cancer survival by targeting ERβ1 [137]. Another intriguing connection between IRE1α and estrogen signaling lies in the poor clinical response of breast cancer samples with high XBP1 levels to the estrogen receptor antagonist prodrug tamoxifen. A compound was developed called STF-083010 to inhibit XBP1 splicing and has been found to restore tamoxifen sensitivity in resistant MCF-7 cells. Coadministration of STF-083010 with tamoxifen gave considerable efficacy in a mouse xenograft model [138].

Conversely, activation of IRE1α may also provide a therapeutic benefit. A compound called LU-102 was developed in order to overcome therapeutic resistance to the proteasome inhibitor bortezomib, caused by a compensatory upregulation of the untargeted proteasomal subunits. Combinatory administration of LU-102 with standard proteasome inhibitors yielded synergistic cytotoxicity via apoptosis induced by IRE1α activity [139]. Bortezomib gives rise to resistant cellular subpopulations in clinical cases although interestingly the coadministration of the demethylating agent 5-azacytidine is able to eliminate much of this resistance [140].

Another mechanism of therapeutically inducing ER stress is the inhibition of histone deacetylase (HDAC) activity, causing the aggregation of misfolded proteins. Particularly in combination with bortezomib, HDAC6 inhibitor ACY-1215 provided a significant delay in tumor growth and prolonged survival in mouse models of lymphoma. Interestingly, the same study illustrated increased XBP1 expression in tumor samples [141]. Redox manipulation provides another avenue of imposing ER stress. The small molecule SK053 was developed in order to target the thioredoxin-thioredoxin reductase system and has considerable efficacy against tumors in mice. By imposing oxidative and ER stress, treatment of tumor cells increases BiP, CHOP, and XBP1s levels, causing apoptosis correlated with the cellular levels of thioredoxin [142]. Another small molecule that appears to target XBP1s is 17#, a small molecule that inhibits tumor growth in vitro and in xenografts synergistically with doxorubicin, etoposide, and 2-deoxy-D-glucose [143].

Coordinated Signaling from PERK, ATF6, and IRE1α

Inhibition of targeted individual branches of the UPR may provide greater clinical efficacy against tumors known to rely on such signaling, In the absence of such information, targeting multiple UPR branches may offer a more useful clinical approach.

Although no pharmacological TMEM33 inhibitors have yet been described, a variety of CREB inhibitors hold promise as a means to target global UPR signaling and sensitivity to apoptosis arising from ER stress [144, 145]. In an ovarian cancer cell line model, β-phenethyl isothiocyanate (PEITC) causes an increase in reactive oxygen species (ROS) and inhibits proliferation by increasing apoptosis via upregulating either PERK or ATF-6 in one model or PERK and IRE1α in another [146]. Likewise, PERK and IRE1α are inhibited by the hepatocellular carcinoma (HCC) therapeutic Sorafenib in HCC cell lines [147]. Conversely, some therapeutic compounds are able to activate each branch of the UPR simultaneously. For example, 3,3-bis(4-hydroxyphenyl)-7-methyl-1,3-dihydro-2H-indol-2-one (BHPI) is a compound that inhibits ERα-controlled gene expression while inducing chronic ER stress via ERα activation and opening of ER calcium channels. The induction of all three branches of UPR signaling induces apoptosis that causes rapid tumor regression among drug-resistant ERα-expressing breast cancer cells in a mouse xenograft model [148].

One way to target global UPR signaling is to target upstream regulators of its activation. In particular, GRP78/BiP may serve as a promising target, particularly for its role in regulating tumor cell autophagy and apoptosis. Inhibition of GRP78/BiP causes increased apoptosis in a mouse model of colon cancer [149]. Pharmacological methods of blocking BiP induction, such as with arctigenin, are especially promising for tumors that depend on UPR to manage proteotoxic stress [92]. Another piece of evidence that GRP78/BiP inhibition has therapeutic value has arisen from studies of drug combination therapy. The coadministration of bortezomib and the antidiabetic agent metformin suppresses the induction of GRP78/BiP, enhances apoptosis, and increases susceptibility to bortezomib in a sample of clinical myeloma tumor cells and xenografts [150].

Several other therapeutics target GRP78/BiP, including docosahexaenoic acid (DHA), which blocks surface GRP78 expression as well as inhibiting PERK [91]. Limited clinical trials have been completed, but targeted combinatory therapy is ongoing in several clinical trials. The antibody PAT-SM6 is another therapeutic that targets cell surface GRP78/BiP expression [90]. Although the primary endpoint of the current PAT-SM6 clinical study is stable disease, that endpoint was reached with the goal of establishing optimal dosage for future clinical trials. The induction of GRP78/BiP is another therapeutic target, inhibited by the preclinical compound versipelostatin [89]. Other therapeutics have been designed to target the ATP-binding domain of GRP78/BiP, including epigallocatechin gallate (EGCG) [93]. Although EGCG has been ineffective in clinical trials targeting smoldering multiple myeloma, it has been effective in therapy to clear HPV and low-grade cervical neoplasia. Future clinical trials could capitalize on the simultaneous inhibition of GRP78/BiP and GRP94 by the administration of pyrvinium [116]. As a counterpart to targeting BiP/GRP78, inhibitors have also been developed that target the other major regulator of PERK and IRE1α, HSP90. One such inhibitor, 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG, or Tanespimycin), has shown limited clinical response in trials to date, but hope remains for the identification of patient subgroups who may be best able to benefit from its effects, especially in combination therapy [106]. One interesting cache of 17-AAG is its selectivity for HSP90 in

tumor cells, in which the protein is uniquely found in multichaperone complexes that have selectively high affinity for 17-AAG [151].

The Future of Cancer Intervention via UPR-Modulatory Drugs

Pharmacological interventions in tumor cell ER function are advancing rapidly. Advances in tumor cell targeting are being advanced particularly by the development of immunogenic therapies. Current therapeutics illustrate the tumor specificity of such therapies, including ER stress-associated anthracyclin induction of cell surface calreticulin expression, important for tumor cell phagocytosis by dendritic cells and immunogenicity in a mouse model [152]. UPR-targeting compounds have some intrinsic specificity for tumor cells given the high levels of ER stress found in tumor cells, thus making UPR inhibition pro-apoptotic in both tumor cells in general and specifically in secretory cells such as those in multiple myeloma [153]. One salient example of tumor cell specificity is the cell-surface expression of BiP found only in tumor cells, giving BiP inhibitors high tumor specificity [52].

However, the limitations of such approaches principally arise from the outgrowth of resistant tumor subpopulations. Resistance to drugs can be caused by factors including modification of target proteins, increased degradation or export of drug molecules, or amplification of cellular machinery that compensates for the targeted molecular signaling. The modification of target proteins can either occur at the transcriptional level via mutations or at the post-translational level. Resistance can be combated by combination therapy, such as the inhibition of PERK in radioresistant hypoxic tumor cells [154]. Combination therapy can be tailored to overcome resistance to a range of therapies, such as oncolytic virus resistance in glioblastoma cells that can be overcome by the inhibition of IRE1α [155].

There are many UPR-related phenomena for which ongoing therapeutic development may be effective. For example, UPR signaling can stimulate inflammation via NF-κB, whose inhibition in metastatic cancer can cause inflammatory tumor growth to give way to inflammation-promoted regression [156]. Although exclusive targeting of NF-κB may be therapeutically limited, drugs such as bortezomib have shown efficacy partly through such inhibition of inflammatory factors such as NF-κB [157]. Moreover, drugs in clinical trials such as AUY922 are able to induce anti-tumorigenic apoptosis via RAF-1 inhibition while inhibiting inflammation via NF-κB [110].

While therapy is able to manage cancer cases, cancer prevention can limit its development by guiding individuals toward anti-tumorigenic lifestyle choices. For example, cigarette smoke induces considerable UPR dysregulation both in vivo and in clinical cases, and decreasing exposure would mitigate the tumorigenic consequences [158]. Likewise, the modification of diet can allow individuals to manage their cancer risk by including compounds such as epigallocatechin gallate, a polyphenol found in green tea that has considerable anticancer properties [159] and targets the ATP-binding domain of BiP [93]. Implementing cancer prevention strategies that leverage our developing understanding of UPR in cancer will allow us to

limit cancer incidence. Meanwhile, thorough characterization of the mechanisms by which cancer cells are able to exploit UPR signaling will provide opportunities to better target the diversity of clinical cases that arise.

References

1. Sevier CS, Kaiser CA. Formation and transfer of disulphide bonds in living cells. Nat Rev Mol Cell Biol. 2002;3:836–47.
2. van Anken E, Braakman I. Versatility of the endoplasmic reticulum protein folding factory. Crit Rev Biochem Mol Biol. 2005;40:191–228.
3. Rao RV, Bredesen DE. Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. Curr Opin Cell Biol. 2004;16:653–62.
4. Lee AS, Delegeane AM, Baker V, Chow PC. Transcriptional regulation of two genes specifically induced by glucose starvation in a hamster mutant fibroblast cell line. J Biol Chem. 1983;258:597–603.
5. Munro S, Pelham HR. An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell. 1986;46:291–300.
6. Gething MJ, Sambrook J. Protein folding in the cell. Nature. 1992;355:33–45.
7. Haas IG, Wabl M. Immunoglobulin heavy chain binding protein. Nature. 1983;306:387–9.
8. Bole DG, Hendershot LM, Kearney JF. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. J Cell Biol. 1986;102:1558–66.
9. Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P. Endoplasmic reticulum stress sensing in the unfolded protein response. Cold Spring Harb Perspect Biol. 2013;5:a013169.
10. Karali E, Bellou S, Stellas D, Klinakis A, Murphy C, Fotsis T. VEGF Signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. Mol Cell. 2014;54:559–72.
11. Scheuner D, Patel R, Wang F, Lee K, Kumar K, Wu J, Nilsson A, Karin M, Kaufman RJ. Double-stranded RNA-dependent protein kinase phosphorylation of the alpha-subunit of eukaryotic translation initiation factor 2 mediates apoptosis. J Biol Chem. 2006;281:21458–68.
12. Sattlegger E, Hinnebusch AG. Separate domains in GCN1 for binding protein kinase GCN2 and ribosomes are required for GCN2 activation in amino acid-starved cells. EMBO J. 2000;19(23):6622–33.
13. Burwick N, Zhang M, Puente P, Azab AK, Hyun TS, Ruiz-Gutierrez M, Sanchez-Bonilla M, Nakamura T, Delrow JJ, MacKay VL, Shimamura A. The eIF2-alpha kinase HRI is a novel therapeutic target in multiple myeloma. Leuk Res. 2017;55:23–32.
14. Andruska N, Zheng X, Yang X, Helferich WG, Shapiro DJ. Anticipatory estrogen activation of the unfolded protein response is linked to cell proliferation and poor survival in estrogen receptor alpha-positive breast cancer. Oncogene. 2015;34:3760–9.
15. Denoyelle C, Abou-Rjaily G, Bezrookove V, Verhaegen M, Johnson TM, Fullen DR, Pointer JN, Gruber SB, Su LD, Nikiforov MA, et al. Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. Nat Cell Biol. 2006;8:1053–63.
16. Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, Qiu B, Zhang H, Cerniglia G, Bi M, et al. ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest. 2012;122:4621–34.
17. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, Harding H, Novoa I, Varia M, Raleigh J, et al. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. EMBO J. 2005;24:3470–81.
18. Hosoi T, Inoue Y, Nakatsu K, Matsushima N, Kiyose N, Shimamoto A, Tahara H, Ozawa K. TERT attenuated ER stress-induced cell death. Biochem Biophys Res Commun. 2014;447(2):378–82.

19. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004;306:457–61.
20. Suh DH, Kim MK, Kim HS, Chung HH, Song YS. Unfolded protein response to autophagy as a promising druggable target for anticancer therapy. Ann N Y Acad Sci. 2012;1271:20–32.
21. Kouroku Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H, Ogawa S, Kaufman RJ, Kominami E, Momoi T. ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. Cell Death Differ. 2007;14:230–9.
22. Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, Koumenis C, Harding HP, Ron D, Holcik M, et al. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol Cell Biol. 2006;26:9517–32.
23. Wang Y, Alam GN, Ning Y, Visioli F, Dong Z, Nor JE, Polverini PJ. The unfolded protein response induces the angiogenic switch in human tumor cells through the PERK/ATF4 pathway. Cancer Res. 2012;72:5396–406.
24. Drogat B, Auguste P, Nguyen DT, Bouchecareilh M, Pineau R, Nalbantoglu J, Kaufman RJ, Chevet E, Bikfalvi A, Moenner M. IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. Cancer Res. 2007;67:6700–7.
25. Auf G, Jabouille A, Guerit S, Pineau R, Delugin M, Bouchecareilh M, Magnin N, Favereaux A, Maitre M, Gaiser T, et al. Inositol-requiring enzyme 1alpha is a key regulator of angiogenesis and invasion in malignant glioma. Proc Natl Acad Sci U S A. 2010;107:15553–8.
26. Dejeans N, Pluquet O, Lhomond S, Grise F, Bouchecareilh M, Juin A, Meynard-Cadars M, Bidaud-Meynard A, Gentil C, Moreau V, et al. Autocrine control of glioma cells adhesion and migration through IRE1alpha-mediated cleavage of SPARC mRNA. J Cell Sci. 2012;125:4278–87.
27. Binet F, Mawambo G, Sitaras N, Tetreault N, Lapalme E, Favret S, Cerani A, Leboeuf D, Tremblay S, Rezende F, et al. Neuronal ER stress impedes myeloid-cell-induced vascular regeneration through IRE1alpha degradation of netrin-1. Cell Metab. 2013;17:353–71.
28. Bartkowiak K, Kwiatkowski M, Buck F, Gorges TM, Nilse L, Assmann V, Andreas A, Muller V, Wikman H, Riethdorf S, et al. Disseminated tumor cells persist in the bone marrow of breast cancer patients through sustained activation of the unfolded protein response. Cancer Res. 2015;75:5367–77.
29. Shen X, Xue Y, Si Y, Wang Q, Wang Z, Yuan J, Zhang X. The unfolded protein response potentiates epithelial-to-mesenchymal transition (EMT) of gastric cancer cells under severe hypoxic conditions. Med Oncol. 2015;32:447.
30. Mahadevan NR, Rodvold J, Sepulveda H, Rossi S, Drew AF, Zanetti M. Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. Proc Natl Acad Sci U S A. 2011;108:6561–6.
31. Rodvold JJ, Mahadevan NR, Zanetti M. Immune modulation by ER stress and inflammation in the tumor microenvironment. Cancer Lett. 2016;380(1):227–36.
32. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol. 2000;2:326–32.
33. Hong F, Liu B, Wu B, Morrell J, Roth B, Davies C, Sun S, Diehl AJ, Li Z. CNPY2 is a key initiator of the PERK-CHOP pathway of the unfolded protein response. Nat Struc Mol Biol. 2017;24(10):834–9.
34. Siekierka J, Manne V, Ochoa S. Mechanism of translational control by partial phosphorylation of the alpha subunit of eukaryotic initiation factor 2. Proc Natl Acad Sci U S A. 1984;81:352–6.
35. Lin JH, Li H, Yasumura D, Cohen HR, Zhang C, Panning B, Shokat KM, Lavail MM, Walter P. IRE1 signaling affects cell fate during the unfolded protein response. Science. 2007;318:944–9.
36. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016;529:326–35.

37. Yaman I, Fernandez J, Liu H, Caprara M, Komar AA, Koromilas AE, Zhou L, Snider MD, Scheuner D, Kaufman RJ, et al. The zipper model of translational control: a small upstream ORF is the switch that controls structural remodeling of an mRNA leader. Cell. 2003;113:519–31.
38. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell. 2000;6:1099–108.
39. Palam LR, Baird TD, Wek RC. Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. J Biol Chem. 2011;286:10939–49.
40. Pettersen EO, Ebbesen P, Gieling RG, Williams KJ, Dubois L, Lambin P, Ward C, Meehan J, Kunkler IH, Langdon SP, et al. Targeting tumour hypoxia to prevent cancer metastasis. From biology, biosensing and technology to drug development: the METOXIA consortium. J Enzyme Inhib Med Chem. 2015;30:689–721.
41. Song B, Scheuner D, Ron D, Pennathur S, Kaufman RJ. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. J Clin Invest. 2008;118:3378–89.
42. Chitnis NS, Pytel D, Bobrovnikova-Marjon E, Pant D, Zheng H, Maas NL, Frederick B, Kushner JA, Chodosh LA, Koumenis C, et al. miR-211 is a prosurvival microRNA that regulates chop expression in a PERK-dependent manner. Mol Cell. 2012;48:353–64.
43. Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, et al. ER stress triggers apoptosis by activating BH3-only protein Bim. Cell. 2007;129:1337–49.
44. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep. 2006;7:880–5.
45. Hou X, Liu Y, Liu H, Chen X, Liu M, Che H, Guo F, Wang C, Zhang D, Wu J, et al. PERK silence inhibits glioma cell growth under low glucose stress by blockage of p-AKT and subsequent HK2's mitochondria translocation. Sci Rep. 2015;5:9065.
46. Higgins R, Gendron JM, Rising L, Mak R, Webb K, Kaiser SE, Zuzow N, Riviere P, Yang B, Fenech E, et al. The unfolded protein response triggers site-specific regulatory ubiquitylation of 40S ribosomal proteins. Mol Cell. 2015;59:35–49.
47. Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, Zhang F, Ye J, Koumenis C, Cavener D, Diehl JA. PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. Oncogene. 2010;29:3881–95.
48. Huber AL, Lebeau J, Guillaumot P, Petrilli V, Malek M, Chilloux J, Fauvet F, Payen L, Kfoury A, Renno T, et al. p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows malignant progression upon low glucose. Mol Cell. 2013;49:1049–59.
49. Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, Zhong Z, Valasek MA, Seki E, Hidalgo J, et al. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. Cancer Cell. 2014;26:331–43.
50. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, Goldstein JL. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell. 2000;6:1355–64.
51. Guan D, Wang H, Li VE, Xu Y, Yang M, Shen Z. N-glycosylation of ATF6beta is essential for its proteolytic cleavage and transcriptional repressor function to ATF6alpha. J Cell Biochem. 2009;108:825–31.
52. Arap MA, Lahdenranta J, Mintz PJ, Hajitou A, Sarkis AS, Arap W, Pasqualini R. Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands. Cancer Cell. 2004;6:275–84.
53. Arai M, Kondoh N, Imazeki N, Hada A, Hatsuse K, Kimura F, Matsubara O, Mori K, Wakatsuki T, Yamamoto M. Transformation-associated gene regulation by ATF6alpha during hepatocarcinogenesis. FEBS Lett. 2006;580:184–90.
54. Wu X, Xin Z, Zhang W, Zheng S, Wu J, Chen K, Wang H, Zhu X, Li Z, Duan Z, et al. A missense polymorphism in ATF6 gene is associated with susceptibility to hepatocellular carcinoma probably by altering ATF6 level. Int J Cancer. 2014;135:61–8.

55. Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res. 2007;67:3496–9.
56. Gupta S, Deepti A, Deegan S, Lisbona F, Hetz C, Samali A. HSP72 protects cells from ER stress-induced apoptosis via enhancement of IRE1alpha-XBP1 signaling through a physical interaction. PLoS Biol. 2010;8:e1000410.
57. Marcu MG, Doyle M, Bertolotti A, Ron D, Hendershot L, Neckers L. Heat shock protein 90 modulates the unfolded protein response by stabilizing IRE1alpha. Mol Cell Biol. 2002;22:8506–13.
58. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature. 2002;415:92–6.
59. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001;107:881–91.
60. Guo FJ, Liu Y, Zhou J, Luo S, Zhao W, Li X, Liu C. XBP1S protects cells from ER stress-induced apoptosis through Erk1/2 signaling pathway involving CHOP. Histochem Cell Biol. 2012;138:447–60.
61. Marza E, Taouji S, Barroso K, Raymond AA, Guignard L, Bonneu M, Pallares-Lupon N, Dupuy JW, Fernandez-Zapico ME, Rosenbaum J, et al. Genome-wide screen identifies a novel p97/CDC-48-dependent pathway regulating ER-stress-induced gene transcription. EMBO Rep. 2015;16:332–40.
62. Li H, Korennykh AV, Behrman SL, Walter P. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proc Natl Acad Sci U S A. 2010;107:16113–8.
63. Yoshida H, Uemura A, Mori K. pXBP1(U), a negative regulator of the unfolded protein response activator pXBP1(S), targets ATF6 but not ATF4 in proteasome-mediated degradation. Cell Struct Funct. 2009;34:1–10.
64. Mishiba K, Nagashima Y, Suzuki E, Hayashi N, Ogata Y, Shimada Y, Koizumi N. Defects in IRE1 enhance cell death and fail to degrade mRNAs encoding secretory pathway proteins in the Arabidopsis unfolded protein response. Proc Natl Acad Sci U S A. 2013;110:5713–8.
65. Tirasophon W, Lee K, Callaghan B, Welihinda A, Kaufman RJ. The endoribonuclease activity of mammalian IRE1 autoregulates its mRNA and is required for the unfolded protein response. Genes Dev. 2000;14:2725–36.
66. Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, Truitt M, McManus MT, Ruggero D, Goga A, et al. IRE1alpha cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. Science. 2012;338:818–22.
67. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 2000;287:664–6.
68. Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravallese EM, Friend D, Grusby MJ, Alt F, Glimcher LH. Plasma cell differentiation requires the transcription factor XBP-1. Nature. 2001;412:300–7.
69. Zhang K, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. The unfolded protein response sensor IRE1alpha is required at 2 distinct steps in B cell lymphopoiesis. J Clin Invest. 2005;115:268–81.
70. Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, Zheng M, Mani M, Henderson J, Pinkus GS, et al. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell. 2007;11:349–60.
71. Leung-Hagesteijn C, Erdmann N, Cheung G, Keats JJ, Stewart AK, Reece DE, Chung KC, Tiedemann RE. Xbp1s-negative tumor B cells and pre-plasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma. Cancer Cell. 2013;24:289–304.
72. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, et al. Patterns of somatic mutation in human cancer genomes. Nature. 2007;446:153–8.

73. Niederreiter L, Fritz TM, Adolph TE, Krismer AM, Offner FA, Tschurtschenthaler M, Flak MB, Hosomi S, Tomczak MF, Kaneider NC, et al. ER stress transcription factor Xbp1 suppresses intestinal tumorigenesis and directs intestinal stem cells. J Exp Med. 2013;210:2041–56.
74. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature. 2014;508:103–7.
75. Jiang H, Zou J, Zhang H, Fu W, Zeng T, Huang H, Zhou F, Hou J. Unfolded protein response inducers tunicamycin and dithiothreitol promote myeloma cell differentiation mediated by XBP-1. Clin Exp Med. 2015;15:85–96.
76. Yeung BH, Kwan BW, He QY, Lee AS, Liu J, Wong AS. Glucose-regulated protein 78 as a novel effector of BRCA1 for inhibiting stress-induced apoptosis. Oncogene. 2008;27:6782–9.
77. Kikuchi D, Tanimoto K, Nakayama K. CREB is activated by ER stress and modulates the unfolded protein response by regulating the expression of IRE1alpha and PERK. Biochem Biophys Res Commun. 2016;469:243–50.
78. Sakabe I, Hu R, Jin L, Clarke R, Kasid UN. TMEM33: a new stress-inducible endoplasmic reticulum transmembrane protein and modulator of the unfolded protein response signaling. Breast Cancer Res Treat. 2015;153:285–97.
79. Tay KH, Luan Q, Croft A, Jiang CC, Jin L, Zhang XD, Tseng HY. Sustained IRE1 and ATF6 signaling is important for survival of melanoma cells undergoing ER stress. Cell Signal. 2014;26:287–94.
80. Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H. TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. EMBO J. 2005;24:1243–55.
81. Hamanaka RB, Bennett BS, Cullinan SB, Diehl JA. PERK and GCN2 contribute to eIF2alpha phosphorylation and cell cycle arrest after activation of the unfolded protein response pathway. Mol Biol Cell. 2005;16:5493–501.
82. Ghosh R, Wang L, Wang ES, Perera BG, Igbaria A, Morita S, Prado K, Thamsen M, Caswell D, Macias H, et al. Allosteric inhibition of the IRE1alpha RNase preserves cell viability and function during endoplasmic reticulum stress. Cell. 2014;158:534–48.
83. Teske BF, Wek SA, Bunpo P, Cundiff JK, McClintick JN, Anthony TG, Wek RC. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Mol Biol Cell. 2011;22:4390–405.
84. Sheng X, Arnoldussen YJ, Storm M, Tesikova M, Nenseth HZ, Zhao S, Fazli L, Rennie P, Risberg B, Waehre H, et al. Divergent androgen regulation of unfolded protein response pathways drives prostate cancer. EMBO Mol Med. 2015;7:788–801.
85. Bustany S, Cahu J, Guardiola P, Sola B. Cyclin D1 sensitizes myeloma cells to endoplasmic reticulum stress-mediated apoptosis by activating the unfolded protein response pathway. BMC Cancer. 2015;15:262.
86. Pagliarini V, Giglio P, Bernardoni P, De Zio D, Fimia GM, Piacentini M, Corazzari M. Downregulation of E2F1 during ER stress is required to induce apoptosis. J Cell Sci. 2015;128:1166–79.
87. Obeng EA, Carlson LM, Gutman DM, Harrington WJ Jr, Lee KP, Boise LH. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood. 2006;107:4907–16.
88. Magnaghi P, D'Alessio R, Valsasina B, Avanzi N, Rizzi S, Asa D, Gasparri F, Cozzi L, Cucchi U, Orrenius C, et al. Covalent and allosteric inhibitors of the ATPase VCP/p97 induce cancer cell death. Nat Chem Biol. 2013;9:548–56.
89. Park HR, Tomida A, Sato S, Tsukumo Y, Yun J, Yamori T, Hayakawa Y, Tsuruo T, Shin-ya K. Effect on tumor cells of blocking survival response to glucose deprivation. J Natl Cancer Inst. 2004;96:1300–10.
90. Rosenes Z, Mok YF, Yang S, Griffin MD, Mulhern TD, Hatters DM, Hensel F, Howlett GJ. Simultaneous binding of the anti-cancer IgM monoclonal antibody PAT-SM6 to low density lipoproteins and GRP78. PLoS One. 2013;8:e61239.

91. Fasano E, Serini S, Piccioni E, Toesca A, Monego G, Cittadini AR, Ranelletti FO, Calviello G. DHA induces apoptosis by altering the expression and cellular location of GRP78 in colon cancer cell lines. Biochim Biophys Acta. 2012;1822:1762–72.
92. Yao X, Zhu F, Zhao Z, Liu C, Luo L, Yin Z. Arctigenin enhances chemosensitivity of cancer cells to cisplatin through inhibition of the STAT3 signaling pathway. J Cell Biochem. 2011;112:2837–49.
93. Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res. 2007;67:9809–16.
94. Guan M, Fousek K, Chow WA. Nelfinavir inhibits regulated intramembrane proteolysis of sterol regulatory element binding protein-1 and activating transcription factor 6 in castration-resistant prostate cancer. FEBS J. 2012;279:2399–411.
95. Gupta SV, Hertlein E, Lu Y, Sass EJ, Lapalombella R, Chen TL, Davis ME, Woyach JA, Lehman A, Jarjoura D, et al. The proteasome inhibitor carfilzomib functions independently of p53 to induce cytotoxicity and an atypical NF-kappaB response in chronic lymphocytic leukemia cells. Clin Cancer Res. 2013;19:2406–19.
96. Tian Z, Zhao JJ, Tai YT, Amin SB, Hu Y, Berger AJ, Richardson P, Chauhan D, Anderson KC. Investigational agent MLN9708/2238 targets tumor-suppressor miR33b in MM cells. Blood. 2012;120:3958–67.
97. Millward M, Price T, Townsend A, Sweeney C, Spencer A, Sukumaran S, Longenecker A, Lee L, Lay A, Sharma G, et al. Phase 1 clinical trial of the novel proteasome inhibitor marizomib with the histone deacetylase inhibitor vorinostat in patients with melanoma, pancreatic and lung cancer based on in vitro assessments of the combination. Investig New Drugs. 2012;30:2303–17.
98. Jin HR, Zhao J, Zhang Z, Liao Y, Wang CZ, Huang WH, Li SP, He TC, Yuan CS, Du W. The antitumor natural compound falcarindiol promotes cancer cell death by inducing endoplasmic reticulum stress. Cell Death Dis. 2012;3:e376.
99. Baritaki S, Yeung K, Palladino M, Berenson J, Bonavida B. Pivotal roles of snail inhibition and RKIP induction by the proteasome inhibitor NPI-0052 in tumor cell chemoimmunosensitization. Cancer Res. 2009;69:8376–85.
100. Fang HT, Zhang B, Pan XF, Gao L, Zhen T, Zhao HX, Ma L, Xie J, Liu Z, Yu XJ, et al. Bortezomib interferes with C-KIT processing and transforms the t(8;21)-generated fusion proteins into tumor-suppressing fragments in leukemia cells. Proc Natl Acad Sci U S A. 2012;109:2521–6.
101. Suraweera A, Munch C, Hanssum A, Bertolotti A. Failure of amino acid homeostasis causes cell death following proteasome inhibition. Mol Cell. 2012;48:242–53.
102. Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, Li WH, Heerding DA, Minthorn E, Mencken T, et al. Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-p yrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J Med Chem. 2012;55:7193–207.
103. Ray A, Vasudevan S, Sengupta S. 6-Shogaol inhibits breast cancer cells and stem cell-like spheroids by modulation of notch signaling pathway and induction of autophagic cell death. PLoS One. 2015;10:e0137614.
104. Krishnamoorthy J, Rajesh K, Mirzajani F, Kesoglidou P, Papadakis AI, Koromilas AE. Evidence for eIF2alpha phosphorylation-independent effects of GSK2656157, a novel catalytic inhibitor of PERK with clinical implications. Cell Cycle. 2014;13:801–6.
105. Chan KC, Ting CM, Chan PS, Lo MC, Lo KW, Curry JE, Smyth T, Lee AW, Ng WT, Tsao GS, et al. A novel Hsp90 inhibitor AT13387 induces senescence in EBV-positive nasopharyngeal carcinoma cells and suppresses tumor formation. Mol Cancer. 2013;12:128.
106. Jhaveri K, Taldone T, Modi S, Chiosis G. Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers. Biochim Biophys Acta. 2012;1823:742–55.

107. Pacey S, Gore M, Chao D, Banerji U, Larkin J, Sarker S, Owen K, Asad Y, Raynaud F, Walton M, et al. A phase II trial of 17-allylamino, 17-demethoxygeldanamycin (17-AAG, tanespimycin) in patients with metastatic melanoma. Investig New Drugs. 2012;30:341–9.
108. Reddy N, Voorhees PM, Houk BE, Brega N, Hinson JM Jr, Jillela A. Phase I trial of the HSP90 inhibitor PF-04929113 (SNX5422) in adult patients with recurrent, refractory hematologic malignancies. Clin Lymphoma Myeloma Leuk. 2013;13:385–91.
109. Xiang L, Gilkes DM, Chaturvedi P, Luo W, Hu H, Takano N, Liang H, Semenza GL. Ganetespib blocks HIF-1 activity and inhibits tumor growth, vascularization, stem cell maintenance, invasion, and metastasis in orthotopic mouse models of triple-negative breast cancer. J Mol Med. 2014;92:151–64.
110. Walsby EJ, Lazenby M, Pepper CJ, Knapper S, Burnett AK. The HSP90 inhibitor NVP-AUY922-AG inhibits the PI3K and IKK signalling pathways and synergizes with cytarabine in acute myeloid leukaemia cells. Br J Haematol. 2013;161:57–67.
111. Qu Z, Wang S, Teng R, Yi X. PU-H71 effectively induces degradation of IkappaB kinase beta in the presence of TNF-alpha. Mol Cell Biochem. 2014;386:135–42.
112. Stenderup K, Rosada C, Gavillet B, Vuagniaux G, Dam TN. Debio 0932, a new oral Hsp90 inhibitor, alleviates psoriasis in a xenograft transplantation model. Acta Derm Venereol. 2014;94:672–6.
113. Haarberg HE, Paraiso KH, Wood E, Rebecca VW, Sondak VK, Koomen JM, Smalley KS. Inhibition of Wee1, AKT, and CDK4 underlies the efficacy of the HSP90 inhibitor XL888 in an in vivo model of NRAS-mutant melanoma. Mol Cancer Ther. 2013;12:901–12.
114. Roue G, Perez-Galan P, Mozos A, Lopez-Guerra M, Xargay-Torrent S, Rosich L, Saborit-Villarroya I, Normant E, Campo E, Colomer D. The Hsp90 inhibitor IPI-504 overcomes bortezomib resistance in mantle cell lymphoma in vitro and in vivo by down-regulation of the prosurvival ER chaperone BiP/Grp78. Blood. 2011;117:1270–9.
115. Ghobrial IM, Campigotto F, Murphy TJ, Boswell EN, Banwait R, Azab F, Chuma S, Kunsman J, Donovan A, Masood F, et al. Results of a phase 2 trial of the single-agent histone deacetylase inhibitor panobinostat in patients with relapsed/refractory Waldenstrom macroglobulinemia. Blood. 2013;121:1296–303.
116. Yu DH, Macdonald J, Liu G, Lee AS, Ly M, Davis T, Ke N, Zhou D, Wong-Staal F, Li QX. Pyrvinium targets the unfolded protein response to hypoglycemia and its anti-tumor activity is enhanced by combination therapy. PLoS One. 2008;3:e3951.
117. MacKenzie MJ, Ernst S, Johnson C, Winquist E. A phase I study of temsirolimus and metformin in advanced solid tumours. Investig New Drugs. 2012;30:647–52.
118. Bouchecareilh M, Higa A, Fribourg S, Moenner M, Chevet E. Peptides derived from the bifunctional kinase/RNase enzyme IRE1alpha modulate IRE1alpha activity and protect cells from endoplasmic reticulum stress. FASEB J. 2011;25:3115–29.
119. Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, Solow-Cordero DE, Bouley DM, Offner F, Niwa M, et al. Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. Blood. 2011;117:1311–4.
120. Mimura N, Fulciniti M, Gorgun G, Tai YT, Cirstea D, Santo L, Hu Y, Fabre C, Minami J, Ohguchi H, et al. Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. Blood. 2012;119:5772–81.
121. Kawamura T, Tashiro E, Shindo K, Imoto M. SAR study of a novel triene-ansamycin group compound, quinotrierixin, and related compounds, as inhibitors of ER stress-induced XBP1 activation. J Antibiot. 2008;61:312–7.
122. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, Silverman RH, Neubert TA, Baxendale IR, Ron D, et al. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. Proc Natl Acad Sci U S A. 2012;109:E869–78.
123. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol. 2003;23:7448–59.

124. Chou TF, Brown SJ, Minond D, Nordin BE, Li K, Jones AC, Chase P, Porubsky PR, Stoltz BM, Schoenen FJ, et al. Reversible inhibitor of p97, DBeQ, impairs both ubiquitin-dependent and autophagic protein clearance pathways. Proc Natl Acad Sci U S A. 2011;108:4834–9.
125. Chou TF, Li K, Frankowski KJ, Schoenen FJ, Deshaies RJ. Structure-activity relationship study reveals ML240 and ML241 as potent and selective inhibitors of p97 ATPase. ChemMedChem. 2013;8:297–312.
126. Brem GJ, Mylonas I, Bruning A. Eeyarestatin causes cervical cancer cell sensitization to bortezomib treatment by augmenting ER stress and CHOP expression. Gynecol Oncol. 2013;128:383–90.
127. Jin X, Li F, Zheng X, Liu Y, Hirayama R, Liu X, Li P, Zhao T, Dai Z, Li Q. Carbon ions induce autophagy effectively through stimulating the unfolded protein response and subsequent inhibiting Akt phosphorylation in tumor cells. Sci Rep. 2015;5:13815.
128. Joo JH, Ueda E, Bortner CD, Yang XP, Liao G, Jetten AM. Farnesol activates the intrinsic pathway of apoptosis and the ATF4-ATF3-CHOP cascade of ER stress in human T lymphoblastic leukemia Molt4 cells. Biochem Pharmacol. 2015;97:256–68.
129. Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, Stanley TB, Sanders B, Goetz A, Gaul N, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013;73:1993–2002.
130. Hussmann M, Janke K, Kranz P, Neumann F, Mersch E, Baumann M, Goepelt K, Brockmeier U, Metzen E. Depletion of the thiol oxidoreductase ERp57 in tumor cells inhibits proliferation and increases sensitivity to ionizing radiation and chemotherapeutics. Oncotarget. 2015;6:39247–61.
131. Fribley AM, Miller JR, Brownell AL, Garshott DM, Zeng Q, Reist TE, Narula N, Cai P, Xi Y, Callaghan MU, et al. Celastrol induces unfolded protein response-dependent cell death in head and neck cancer. Exp Cell Res. 2015;330:412–22.
132. Flaherty DP, Miller JR, Garshott DM, Hedrick M, Gosalia P, Li Y, Milewski M, Sugarman E, Vasile S, Salaniwal S, et al. Discovery of sulfonamidebenzamides as selective apoptotic CHOP pathway activators of the unfolded protein response. ACS Med Chem Lett. 2014;5:1278–83.
133. Sidhu A, Miller JR, Tripathi A, Garshott DM, Brownell AL, Chiego DJ, Arevang C, Zeng Q, Jackson LC, Bechler SA, et al. Borrelidin induces the unfolded protein response in oral cancer cells and chop-dependent apoptosis. ACS Med Chem Lett. 2015;6:1122–7.
134. Leclerc GJ, DeSalvo J, Du J, Gao N, Leclerc GM, Lehrman MA, Lampidis TJ, Barredo JC. Mcl-1 downregulation leads to the heightened sensitivity exhibited by BCR-ABL positive ALL to induction of energy and ER-stress. Leuk Res. 2015;39:1246–54.
135. Guan M, Su L, Yuan YC, Li H, Chow WA. Nelfinavir and nelfinavir analogs block site-2 protease cleavage to inhibit castration-resistant prostate cancer. Sci Rep. 2015;5:9698.
136. Bobak Y, Kurlishchuk Y, Vynnytska-Myronovska B, Grydzuk O, Shuvayeva G, Redowicz MJ, Kunz-Schughart LA, Stasyk O. Arginine deprivation induces endoplasmic reticulum stress in human solid cancer cells. Int J Biochem Cell Biol. 2016;70:29–38.
137. Rajapaksa G, Nikolos F, Bado I, Clarke R, Gustafsson JA, Thomas C. ERbeta decreases breast cancer cell survival by regulating the IRE1/XBP-1 pathway. Oncogene. 2015;34:4130–41.
138. Ming J, Ruan S, Wang M, Ye D, Fan N, Meng Q, Tian B, Huang T. A novel chemical, STF-083010, reverses tamoxifen-related drug resistance in breast cancer by inhibiting IRE1/XBP1. Oncotarget. 2015;6:40692–703.
139. Kraus M, Bader J, Geurink PP, Weyburne ES, Mirabella AC, Silzle T, Shabaneh TB, van der Linden WA, de Bruin G, Haile SR, et al. The novel beta2-selective proteasome inhibitor LU-102 synergizes with bortezomib and carfilzomib to overcome proteasome inhibitor resistance of myeloma cells. Haematologica. 2015;100:1350–60.
140. Adomako A, Calvo V, Biran N, Osman K, Chari A, Paton JC, Paton AW, Moore K, Schewe DM, Aguirre-Ghiso JA. Identification of markers that functionally define a quiescent multiple myeloma cell sub-population surviving bortezomib treatment. BMC Cancer. 2015;15:444.

141. Amengual JE, Johannet P, Lombardo M, Zullo K, Hoehn D, Bhagat G, Scotto L, Jirau-Serrano X, Radeski D, Heinen J, et al. Dual targeting of protein degradation pathways with the selective HDAC6 inhibitor ACY-1215 and bortezomib is synergistic in lymphoma. Clin Cancer Res. 2015;21:4663–75.
142. Muchowicz A, Firczuk M, Wachowska M, Kujawa M, Jankowska-Steifer E, Gabrysiak M, Pilch Z, Klossowski S, Ostaszewski R, Golab J. SK053 triggers tumor cells apoptosis by oxidative stress-mediated endoplasmic reticulum stress. Biochem Pharmacol. 2015;93:418–27.
143. Huang H, Liu H, Liu C, Fan L, Zhang X, Gao A, Hu X, Zhang K, Cao X, Jiang K, et al. Disruption of the unfolded protein response (UPR) by lead compound selectively suppresses cancer cell growth. Cancer Lett. 2015;360:257–68.
144. Arensman MD, Telesca D, Lay AR, Kershaw KM, Wu N, Donahue TR, Dawson DW. The CREB-binding protein inhibitor ICG-001 suppresses pancreatic cancer growth. Mol Cancer Ther. 2014;13:2303–14.
145. Xie F, Li BX, Kassenbrock A, Xue C, Wang X, Qian DZ, Sears RC, Xiao X. Identification of a potent inhibitor of CREB-mediated gene transcription with efficacious in vivo anticancer activity. J Med Chem. 2015;58:5075–87.
146. Hong YH, Uddin MH, Jo U, Kim B, Song J, Suh DH, Kim HS, Song YS. ROS accumulation by PEITC selectively kills ovarian cancer cells via UPR-mediated apoptosis. Front Oncol. 2015;5:167.
147. Houessinon A, Gicquel A, Bochereau F, Louandre C, Nyga R, Godin C, Degonville J, Fournier E, Saidak Z, Drullion C, et al. Alpha-fetoprotein is a biomarker of unfolded protein response and altered proteostasis in hepatocellular carcinoma cells exposed to sorafenib. Cancer Lett. 2016;370:242–9.
148. Andruska ND, Zheng X, Yang X, Mao C, Cherian MM, Mahapatra L, Helferich WG, Shapiro DJ. Estrogen receptor alpha inhibitor activates the unfolded protein response, blocks protein synthesis, and induces tumor regression. Proc Natl Acad Sci U S A. 2015;112:4737–42.
149. Sakitani K, Hirata Y, Hikiba Y, Hayakawa Y, Ihara S, Suzuki H, Suzuki N, Serizawa T, Kinoshita H, Sakamoto K, et al. Inhibition of autophagy exerts anti-colon cancer effects via apoptosis induced by p53 activation and ER stress. BMC Cancer. 2015;15:795.
150. Jagannathan S, Abdel-Malek MA, Malek E, Vad N, Latif T, Anderson KC, Driscoll JJ. Pharmacologic screens reveal metformin that suppresses GRP78-dependent autophagy to enhance the anti-myeloma effect of bortezomib. Leukemia. 2015;29:2184–91.
151. Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, Burrows FJ. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature. 2003;425:407–10.
152. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med. 2007;13:54–61.
153. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer. 2014;14:581–97.
154. Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, van der Kogel AJ, Koritzinsky M, Wouters BG. PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. Proc Natl Acad Sci U S A. 2013;110:4622–7.
155. Mahoney DJ, Lefebvre C, Allan K, Brun J, Sanaei CA, Baird S, Pearce N, Gronberg S, Wilson B, Prakesh M, et al. Virus-tumor interactome screen reveals ER stress response can reprogram resistant cancers for oncolytic virus-triggered caspase-2 cell death. Cancer Cell. 2011;20:443–56.
156. Zagury D, Gallo RC. Anti-cytokine Ab immune therapy: present status and perspectives. Drug Discov Today. 2004;9:72–81.
157. Juvekar A, Manna S, Ramaswami S, Chang TP, Vu HY, Ghosh CC, Celiker MY, Vancurova I. Bortezomib induces nuclear translocation of IκBα resulting in gene-specific suppression of NF-κB-dependent transcription and induction of apoptosis in CTCL. Mol Cancer Res. 2011;9(2):183–94.

158. Zhao H, Yang J, Shan L, Jorgensen ED. Measuring the impact of cigarette smoke on the UPR. Methods Enzymol. 2011;489:147–64.
159. Du GJ, Zhang Z, Wen XD, Yu C, Calway T, Yuan CS, Wang CZ. Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. Nutrients. 2012;4:1679–91.

Chapter 4
ATF4, Hypoxia and Treatment Resistance in Cancer

Dean C. Singleton and Adrian L. Harris

Abstract Oxygen deprivation (hypoxia) is a common feature of tumors that is associated with treatment resistance and poor patient survival. Hypoxia perturbs the oxidative environment within the endoplasmic reticulum (EnR), limiting protein folding capacity. This restriction causes an accumulation of unfolded proteins in the EnR and activation of a stress response pathway, termed the unfolded protein response (UPR). Signals from the UPR culminate in repression of general protein translation. Paradoxically, a small number of transcripts are selectively translated under these conditions. One of these transcripts encodes Activating Transcription Factor 4 (ATF4). In tumors, ATF4 expression is detected in hypoxic and nutrient-deprived regions. ATF4 promotes metabolic homeostasis and cancer cell survival by transcriptionally regulating numerous processes including amino acid uptake, antioxidant biosynthesis, and autophagy. These changes confer ATF4-expressing cells with a multidrug resistance phenotype and the ability to tolerate adverse stresses of the tumor microenvironment. However, under conditions of persistent and unresolved stress, ATF4 transcriptional reprogramming becomes pro-apoptotic. Therapeutic modulators of ATF4 signaling have the potential to modify these properties by diminishing adaptive phenotypes in cancer cells. Reprogramming cancer cells in this way can improve tumor sensitivity to anticancer treatments including chemotherapy, immunotherapy, and radiotherapy.

Keywords Autophagy · ATF4 · EnR stress · Unfolded protein response · Hypoxia · PERK · GCN2

D. C. Singleton (✉)
Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand
e-mail: d.singleton@auckland.ac.nz

A. L. Harris
Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_4

Tumor Hypoxia

The tumor microenvironment is characterized by transient fluctuations in oxygen concentration [1]. Areas of oxygen deprivation (hypoxia) develop because the immature and disorganized tumor microvasculature is unable to deliver sufficient oxygen to meet the metabolic demands of the tumor. Hypoxia is commonly observed in solid human tumors. For example, hypoxic regions with $pO_2 < 2.5$ mmHg (equivalent to ~0.3% O_2) are frequent in locally advanced breast tumors but are not detected in normal breast tissue [2, 3]. However, the proportion of hypoxic cells ($pO_2 < 2.5$ mmHg) in tumors is highly variable between patients and can range from 0 to 97% [4]. Hypoxic cancer cells stimulate tumor growth by secreting pro-angiogenic factors, particularly Vascular Endothelial Growth Factor A (VEGFA). In addition, hypoxic cancer cells display enhanced malignant phenotypes including tumor initiation and invasion. Critically, these cells are resistant to killing by radiotherapy and some forms of chemotherapy [3, 5]. Consequently, patients with hypoxic tumors have an increased risk of metastasis and reduced overall survival probability [6–9].

Early reports described hypoxic cells in tumors in perinecrotic regions at distances >150 μm from vessels [10, 11]. These *chronically* hypoxic cells occur because oxygen is consumed as it diffuses away from the blood vessels, resulting in a steep oxygen gradient between oxygenated cells adjacent to the tumor vasculature and severely hypoxic cells in perinecrotic regions (Fig. 4.1a). Hypoxic cells can also exist in closer proximity to the tumor microvasculature following a transient vessel occlusion [12]. These *acutely* hypoxic cells are hypothesized to pose a greater clinical problem than cells that are chronically hypoxic. This is because cells that are transiently hypoxic are likely to be temporarily chemotherapy and radiotherapy resistant, allowing them to survive treatment, and then continue proliferating following re-oxygenation. Furthermore, transcriptionally reprogrammed hypoxic cells that are near tumor vessels pose a higher risk of hematogenous metastasis than chronically hypoxic cells that are confined to perinecrotic regions that are distal to the vasculature [13, 14].

The spatial heterogeneity in acute hypoxia is also subject to fluctuations over time [15]. Animal models have demonstrated that acute hypoxia occurs in a cyclic manner with rapid changes in the subpopulation of acutely hypoxic cells within the tumor [16]. However, despite the common classification used to divide hypoxic tumor cells into two distinct subtypes, the true pathophysiology of tumor hypoxia is likely to reflect both transient and sustained episodes that range from mild oxygen deprivation to complete anoxia, resulting in heterogeneous biological responses that depend on both extrinsic and intrinsic factors.

The adverse effect of tumor hypoxia on patient survival has motivated the development of strategies to target hypoxic cells in tumors [17]. These include prodrugs designed to undergo selective activation in hypoxic cells and molecularly targeted agents developed to interfere with cellular mechanisms of hypoxic adaptation.

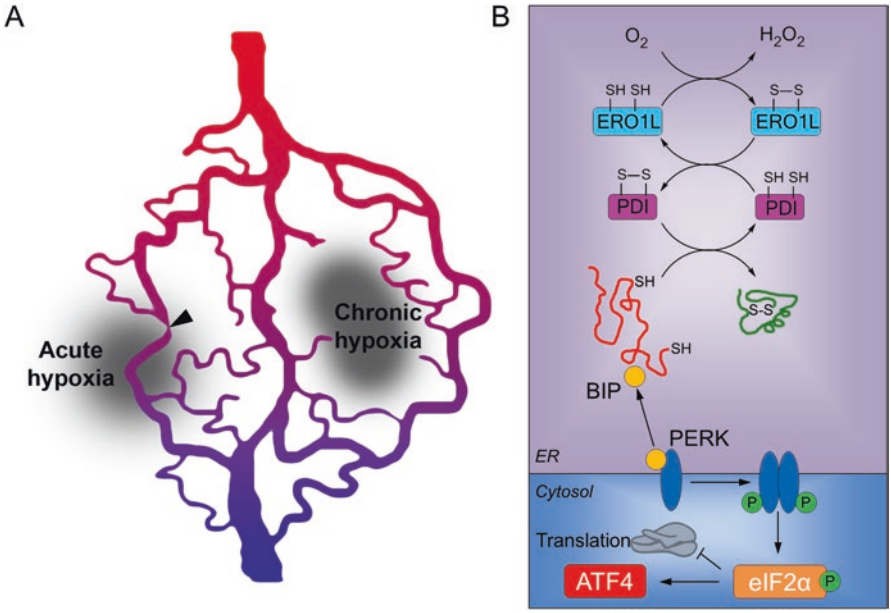

Fig. 4.1 (**a**) Schematic representation of the tumor vasculature demonstrating areas of chronic and acute hypoxia. Regions of the tumor that are beyond the diffusion limits of oxygen are chronically hypoxic. Transient vessel occlusion (arrowhead) results in an acute hypoxic episode in close proximity to the tumor vasculature. (**b**) Oxygen is required for protein folding in the EnR. In this system, protein folding and disulfide bond formation is driven by the enzymes PDI and ERO1L with oxygen acting as the final electron acceptor. Hypoxia limits this reaction resulting in the accumulation of unfolded proteins (red polypeptide). Unfolded proteins sequester the EnR chaperone BIP resulting in the autophosphorylation and activation of PERK. PERK then phosphorylates eIF2α to repress the global rate of protein translation. However, under these conditions ATF4 mRNA is selectively translated

Therapeutic Targeting of Tumor Hypoxia

Hypoxia-activated prodrugs (HAPs) represent a promising, clinically advanced strategy to eliminate hypoxic cells. HAPs are designed to be selectively activated in hypoxic cells. This is achieved via an oxygen-sensitive mechanism of activation that relies on one-electron reductases, particularly cytochrome P450 oxidoreductase (POR) [17]. In hypoxia, HAPs undergo metabolism to species that are more potent than the HAP itself. Whereas, in normoxic cells HAPs undergo minimal conversion to cytotoxic species, resulting in negligible cell killing. This difference in cytotoxicity provides therapeutic selectivity for hypoxic tumor cells. A number of promising HAPs have been developed, with some undergoing Phase II/III clinical investigation including Tirapazamine, Evofosfamide, PR-104, and EO9 [17–20].

Confining HAP activation to conditions of low (pathogenic) oxygen (<0.2% O_2) provides an opportunity to exploit hypoxia as a tumor-specific property because the lower ranges of physiological O_2 tension are approximately 3–9% in normal tissues

[21]. This feature of tumor-selective activation may be particularly useful if cytotoxic metabolites released from HAPs are designed with considerable "bystander" properties so that they can diffuse into nearby oxygenated cells to extend the level of tumor killing beyond the hypoxic region and increase the anticancer activity of the HAP [22]. Development of companion methods, including hypoxia imaging using Positron Emission Tomography (PET) will enable prediction of patients with hypoxic, POR-expressing tumors that are most likely to respond to treatment with HAPs [23–25].

Molecular Targeting of Tumor Hypoxia

An alternative strategy to eliminate hypoxic tumor cells is to exploit the molecular vulnerabilities that occur within this subpopulation. This concept has been motivated by a growing understanding of the biological changes that underpin the pro-survival adaptations to hypoxia [26]. Much of this work has focused on inhibiting the Hypoxia Inducible Factor (HIF) family of transcription factors or targeting phenotypic changes that are dependent on expression of HIF-target genes [27]. Although HIF is a challenging protein to inhibit directly, several drugs that prevent HIF transcriptional function have advanced to clinical evaluation, including PX-478 and PT2385 [28]. Indirect strategies have relied on targeting HIF-dependent genes, for example, *CA9*, *LOX*, *GLUT1,* and *RIOK3* or antagonizing phenotypes that are associated with HIF-target genes, for example, reactivation of mitochondrial respiration using dichloroacetate [29–34].

A number of HIF-independent pathways of hypoxic adaptation have been described including AMP-activated protein kinase (AMPK) signaling and the unfolded protein response (UPR) [35]. The UPR has emerged as an important mechanism that promotes tolerance to cell stress resulting from nutrient deprivation, hypoxia, or exposure to pharmacological agents. The UPR acts as a key link between oxygen availability and the rate of protein translation. Notably, several features of the UPR can be targeted with drugs, providing new strategies to eliminate or modify hypoxic cell behavior in tumors, with the potential to complement or enhance the efficacy of current cancer treatment regimens.

The Unfolded Protein Response Is Activated by Severe Hypoxia

Secreted and cell surface proteins undergo folding, glycosylation, disulfide bond formation, and structural maturation in the EnR. To accomplish these processes, EnR function is strictly dependent on the maintenance of a distinct oxidative environment [36, 37]. Severe hypoxia perturbs the redox potential of the EnR resulting

in accumulation of unfolded client proteins in the ER lumen [37]. During disulfide bond formation, an oxidoreductase (ERO1L) and isomerase (PDI) act as a relay system to transfer electrons from the EnR client protein to molecular oxygen (Fig. 4.1b). Disulfide bond formation is crucial for correct protein folding [38], highlighting the obligatory role of oxygen in EnR function. Recent studies have demonstrated that the initial phase of disulfide bond formation that occurs during protein translation can proceed independently of oxygen, whereas post-translational disulfide bond formation and isomerase steps are oxygen dependent [39].

Accumulation of misfolded proteins in the EnR activates the UPR. The UPR acts to resolve EnR stress by increasing the folding capacity of the EnR, suppressing protein translation and by increasing the rate of degradation of misfolded proteins (by EnR-associated degradation or autophagy). The UPR is initiated by three EnR transmembrane proteins: inositol-requiring enzyme 1α (IRE1α; *ERN1*), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK; *EIF2AK3*) and Activating Transcription Factor 6 (ATF6) [40, 41]. Mechanistically, the accumulated misfolded proteins in the EnR lumen displace BIP (GRP78, *HSPA5*), a molecular chaperone, from the luminal domains of IRE1α and PERK, triggering their dimerization, autophosphorylation, and activation [42].

PERK-eIF2α-ATF4 Signaling

Recent structural, biophysical, and cellular analysis suggest that EnR stress causes a transient tetrameric arrangement of PERK luminal domains, and this organization is required for PERK activity [43]. Activated PERK phosphorylates the eukaryotic translation initiation factor 2α (eIF2α) on serine residue 51 [44]. This post-translational modification results in reversible repression of protein translational initiation. Three additional kinases (HRI, PKR, and GCN2) can phosphorylate eIF2α in response to distinct cellular stress events.

During translation initiation the eIF2 complex (composed of α, β, and γ subunits) binds to GTP and the initiator methionyl-tRNA. This ternary complex associates with eIF1, eIF1A, eIF3, and the 40S ribosomal subunit to form the 43S preinitiation complex (PIC). The PIC scans along mRNA that has been unwound by eIF4F and recognizes the AUG start codon, prompting eIF5-dependent GTP hydrolysis [45]. The GDP-bound eIF2 is then released allowing recruitment of the 60S ribosomal subunit and initiation of translation. The guanine nucleotide exchange factor (GEF) eIF2B then catalyzes the exchange of GDP for GTP to recover eIF2-GTP, enabling another round of translation initiation. This replenishing step is a key regulatory stage in the rate of translational initiation. Importantly, the GEF activity of eIF2B is controlled by the phosphorylation state of eIF2α at serine 51. Phosphorylation at this site causes an increase in affinity of eIF2B for eIF2-GDP, reducing the exchange of eIF2-GDP to eIF2-GTP and limiting the rate of translation initiation [46, 47].

Although the global rate of mRNA translation is repressed during conditions of elevated eIF2α phosphorylation, paradoxically, the translation of a number of

mRNA transcripts, including ATF4, is increased [48]. The translational control of ATF4 expression is dependent on two upstream open reading frames (uORFs) in the 5′ region of the *ATF4* mRNA. When eIF2α phosphorylation is low, eIF2-GTP is abundant. Ribosomes translate the 5′ uORF1 and then scan along the *ATF4* mRNA to reinitiate translation efficiently at the downstream uORF2 [49, 50]. The second (inhibitory) uORF2 overlaps with the start codon of the ATF4 ORF and, therefore, translation of the ATF4 ORF is prevented and cellular levels of ATF4 protein remain low. During conditions where eIF2α phosphorylation is elevated, the availability of eIF2-GTP is reduced and the scanning ribosomes take longer to become competent. This delay allows the ribosome to bypass the inhibitory uORF2 and instead reinitiate translation at the ATF4 ORF, resulting in increased levels of ATF4 translation. Once expressed, the ATF4 protein can translocate into the nucleus and transcriptionally regulate a number of genes required for amino acid synthesis and import, autophagy, redox balance, and angiogenesis [51].

This prevailing model of uORF-based *ATF4* translational regulation has recently been refined to include a role for N^6-Methyladenosine (m6A) [52]. Zhou et al. demonstrated an increased enrichment of m6A within the inhibitory uORF2. Cellular stress resulting in phosphorylation of eIF2α reduced the abundance of this modification, in a process catalyzed by ALKBH5-dependent demethylation. Thus, m6A within uORF2 impedes the scanning ribosomes resulting in increased translation of inhibitory uORF2 and reduced translation of the *ATF4* ORF.

DDX3, an RNA-binding protein, was shown to increase phospho-eIF2α-dependent ATF4 translation via interaction with the eIF4F complex, identifying another factor that contributes to the control of ATF4 expression levels [53]. In addition, mTORC1 promotes ATF4 translation through its uORFs, but acts independently of eIF2α phosphorylation status [54]. These recent findings highlight the important work still needed to fully elucidate the mechanism of ATF4 translational control.

In addition to *ATF4*, several other transcripts undergo preferential translation during episodes of increased eIF2α phosphorylation [55]. For example, CHOP *(DDIT3)* and *GADD34*, target genes that are transcriptionally up-regulated by ATF4, are also regulated by a translational mechanism that depends on 5′ inhibitory uORFs [56, 57].

Activity of ATF4 in Hypoxic Cells

ATF4 is a basic region-leucine zipper transcription factor [58, 59]. In vitro exposure to severe hypoxia results in elevated expression of ATF4 [60, 61]. In agreement, ATF4 expression is observed in hypoxic regions of human tumors and levels of ATF4 are elevated in breast cancer, cervical cancer, melanoma, and glioblastoma compared with corresponding normal tissue [62].

Pro-Survival Versus Pro-death Roles of ATF4

ATF4 promotes adaptation and survival during periods of cellular stress and loss of ATF4 results in hypersensitivity to EnR stress generated by hypoxia [62]. However, in situations where the stress is excessive and cannot be resolved, ATF4 acts to promote cell death, with many reports implicating CHOP in this process. Detailed transcriptomic studies have shed more light on this dichotomy of function [63]. Han et al. found that overexpression of ATF4 resulted in transcription of several known ATF4 responsive genes (*ATF3, GADD34, TRIB3*). The level of transcription was markedly increased by co-expression of CHOP, demonstrating that CHOP co-operates with ATF4. In contrast, CHOP overexpression alone had negligible effect on gene expression. ATF4/CHOP responsive genes were largely involved in stimulating protein synthesis, for example, aminoacyl-tRNA synthetase genes. Notably, ATF4 and CHOP did not induce genes that promote apoptosis, suggesting that they do not directly participate in the transcriptional induction of apoptosis.

Chromatin immunoprecipitation sequencing (ChIP-seq) studies showed that ATF4 and CHOP bind at a common site in the proximal promoter region of these genes at a consensus sequence [63]. Furthermore, direct interaction between ATF4 and CHOP was confirmed by co-immunoprecipitation studies. These findings need to be carefully interpreted and considered alongside the temporal kinetics of EnR stress events. Following treatment with pharmacological inducers of EnR stress, cells display a rapid increase in eIF2α phosphorylation, proceeded sequentially by ATF4 expression, then CHOP expression and then apoptosis. The evidence suggests that early events (translational repression and ATF4 expression) are *pro-survival* responses that are directed towards alleviation of EnR stress. In support of this idea, ATF4 alone (in the absence of CHOP) transcriptionally up-regulates target genes involved in amino acid transport and biosynthesis (e.g., *SLC6A9* and *PSAT1*) [63]. In contrast, later events that depend on CHOP are directed towards re-establishment of protein synthesis. ATF4 and CHOP co-operatively induce expression of genes involved in protein translation including aminoacyl-tRNA synthetases and initiation factors (e.g., *WARS* and *EIF5*). In addition, the late up-regulation of GADD34 leads to dephosphorylation of eIF2α and a resumption of protein translation. Where the EnR stress has been resolved, for example, following re-oxygenation of tumor cells, the activity of ATF4/CHOP would promote a return to basal or even elevated levels of protein synthesis leading to enhanced tumor growth, having carried out earlier transient pro-survival functions. In contrast, during conditions of unresolved EnR stress, for example, chronic hypoxia, ATF4/CHOP would transcriptionally induce protein synthesis resulting in further oxidative stress, ATP depletion, and apoptosis. Thus, the microenvironmental heterogeneity in oxygen tension, nutrient availability, and temporal kinetics of stress within the tumor determine whether ATF4 acts in a pro-survival or pro-death capacity.

Phenotypic Reprogramming by ATF4

ATF4 promotes adaptation to cellular stress by transcriptionally up-regulating genes required for autophagy, redox maintenance, amino acid homeostasis, angiogenesis, and metastasis (Fig. 4.2).

Regulation of Autophagy by ATF4

Macroautophagy (hereafter termed autophagy) is a process whereby cytoplasmic macromolecules, protein aggregates, and organelles, including the EnR and mitochondria, are degraded by the lysosome and recycled [64]. All cells maintain a basal level of autophagy to remove damaged and long-lived proteins that are not degraded via the proteasome. During hypoxia the rate of autophagy is increased to promote cell survival. This increase in autophagy enables recycling of amino acids during episodes of stress, but, perhaps most critically, allows for detoxification and removal of proteins and organelles that have been damaged by reactive oxygen species (ROS) [65, 66]. High levels of ROS are generated during cyclic hypoxic exposures

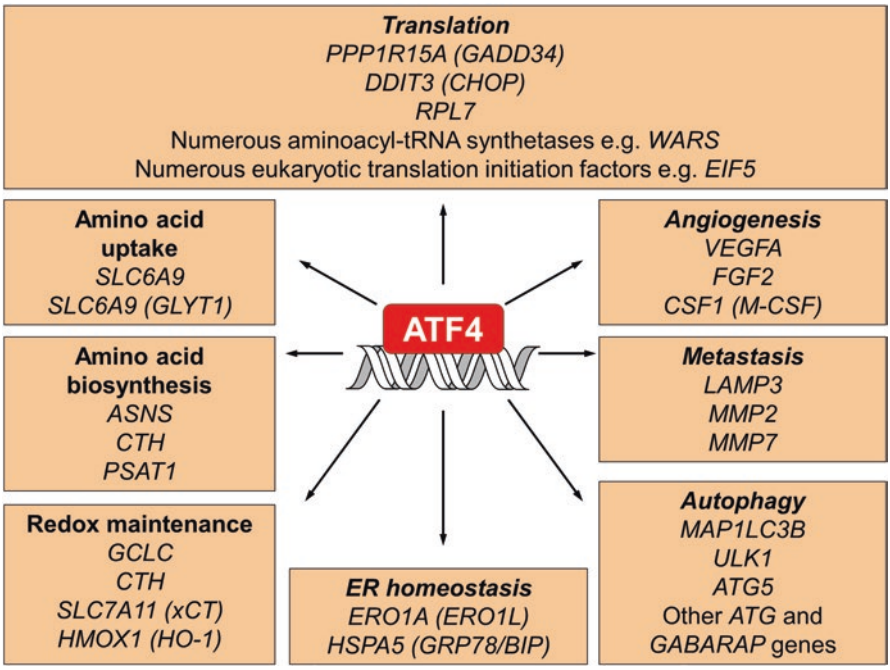

Fig. 4.2 ATF4-dependent processes and examples of target genes that are associated with these processes

[67]. Consequently, inhibitors of autophagy (e.g., choloroquine) sensitize cells to hypoxic stress [66, 68].

ATF4 is an important promoter of autophagy by transcriptionally up-regulating several autophagy-related genes including *MAP1LC3B* (commonly referred to as LC3B) [68, 69]. LC3B is incorporated into autophagosomes during autophagy. During high rates of autophagy LC3B becomes depleted, eventually limiting the rate of autophagy. ATF4-dependent transcription of *MAP1LC3B* helps to maintain LC3B concentrations and sustain high rates of autophagic flux during cellular stress. ATG5, a protein involved in autophagosome elongation, is also induced during EnR stress in an ATF4- and CHOP-dependent manner [69]. Notably, ATF4 also up-regulates ULK1, an inducer of autophagy, in certain cancer cell lines, demonstrating that ATF4 can also transcriptionally promote autophagy initiation [70]. Studies in mouse embryonic fibroblasts (MEFs) have extended the list of autophagy genes that are known to be regulated by Atf4 to include *Atg16l1*, *Atg12*, *Atg3*, *Becn1*, and *Gabarapl2* [71]. Furthermore, Atf4, in combination with Chop, has also been demonstrated to transcriptionally up-regulate *p62* (*Sqstm1*), *Nbr1* and *Atg7* [71].

These studies, and others, underline the important role of ATF4 as a master regulator of autophagy gene transcription [72]. Correspondingly, ATF4 is necessary for autophagy during various states of cell stress, including transformation. Transformation with c-Myc increases cellular rates of protein translation but can also cause apoptosis. Myc-driven protein translation results in higher levels of EnR stress [73]. The PERK-eIF2α-ATF4 pathway supports cyto-protective autophagy in this context. PERK-deficient cells undergo apoptosis following transformation with Myc and PERK is required for the growth of Myc-driven tumors. Similarly, ATF4 is required during transformation of MEFs with H-ras^{V12} and SV40 large T antigen [74]. This work establishes an essential role for the PERK-eIF2α-ATF4 pathway in the transforming activity of oncogenes. Notably, ATF4 can play multiple roles in Myc-driven oncogenesis. Depriving Myc-amplified neuroblastoma cells of glutamine activates the GCN2-eIF2α-ATF4 signaling pathway. In this context, ATF4 promotes cell death by transcriptionally up-regulating *PUMA*, *NOXA*, and *TRIB3* [75].

Role of ATF4 in Redox Maintenance

During hypoxia and other tumor microenvironmental stresses cancer cells can experience supraphysiological levels of ROS [67, 76]. These species cause damage to proteins and organelles resulting in cytotoxicity. ATF4 helps to protect cells from excessive oxidative stress by increasing levels of glutathione (GSH), a key cellular antioxidant. Increased GSH is achieved by transcriptionally up-regulating glycine import (e.g., *GLYT1*) [51], cysteine import (e.g., *SLC7A11* and *SLC1A4*) [77, 78], cysteine availability (e.g., *CTH*) [51, 79], and GSH biosynthesis (e.g., *GCLC*) [80]. Cells that lack ATF4 have lower levels of GSH and greater sensitivity to oxidative stress [79].

Role of ATF4 in Cell Metabolism and Amino Acid Homeostasis

Initial studies using ATF4 knockout MEFs demonstrated impaired cell growth kinetics compared with wild-type counterparts [51]. However, this proliferative defect was rescued by addition of non-essential amino acids (NEAA) and reducing agents, such as β-mercaptoethanol, into the culture medium. Similar effects are observed in cancer cells; ATF4 knockdown reduced cell survival and proliferation and increased apoptosis [81]. These defects were rescued by addition of either NEAA or by asparagine alone or by overexpression of asparagine synthetase (*ASNS*). Furthermore, ATF4 expression was necessary for growth of tumor xenografts [81].

Further work has confirmed the importance of the GCN2-eIF2α-ATF4 pathway as a key sensor of amino acid depletion. Upon activation, ATF4 triggers an adaptive response involving up-regulation of several genes encoding amino acid uptake and synthesis proteins. The in vitro induction of this program has been reported following depletion of several individual amino acids including methionine, asparagine, leucine, tryptophan, serine, glutamine, and cysteine, although the sensitivity to depletion of individual amino acids likely depends on the plasticity of the cellular metabolic environment and is thus cell line- and cell type dependent [82–86]. Induction of the amino acid stress response was particularly notable in conditions where autophagy was impaired and glutamine concentrations were constrained [87].

When serine concentrations are reduced, the GCN2-eIF2α-ATF4 pathway is activated, and this results in the transcription of *PHGDH*, *PSAT1,* and *PSPH* and increased serine biosynthesis [85]. Serine acts to positively regulate Pyruvate Kinase M2 (PKM2) enzyme activity. When serine levels are low, PKM2 activity is repressed leading to an accumulation of glycolytic intermediates that can feed into the serine biosynthetic pathway [88]. Thus, control of serine biosynthesis depends on the concerted activity of PKM2 and ATF4. Maintenance of intracellular serine levels is required to support mTORC1 activity and sustain cell proliferation.

In vivo, fluctuating concentrations of amino acids, particularly glutamine, can occur in the tumor microenvironment, potentially in concurrence with hypoxia and glucose limitation (i.e., ischemia) [89–91]. Activation of the GCN2-eIF2α-ATF4 pathway in response to glutamine deprivation provides an adaptive feedback mechanism to increase amino acid uptake [92]. ATF4 expression also occurs in response to pharmacological glutamine deprivation following treatment with glutaminase inhibitors [93].

A more complex picture of this biological pathway has recently emerged. In *KRAS*-mutant non-small cell lung carcinoma (NSCLC) ATF4 plays a key role in disease biology through regulation of amino acid metabolism [94]. Gwinn et al. demonstrated that KRAS-PI3K-AKT-NRF2 signaling in this context was required for expression of ATF4 in response to cell growth in conditions of physiological glutamine abundance (0.5 mM). Upstream GCN2-phospho-eIF2α signaling was implicated in the elevated expression of ATF4 observed in these conditions. However, further work highlighted

the importance of genetic context in these processes; *KRAS* mutant cells with additional *KEAP1* mutation, leading to NRF2 activation, demonstrated improved survival in conditions of glutamine deprivation when ATF4 was knocked down. Thus, the abundant ATF4 in these cells acts in a pro-apoptotic manner during conditions of amino acid starvation. In contrast, *KRAS* mutant/*KEAP1* WT or *KRAS* WT/*KEAP1* WT cells did not benefit from ATF4 knockdown during conditions of nutrient stress. However, the pro-survival effect of ATF4 loss in the *KRAS* mutant/*KEAP1* mutant context was not seen in xenograft models, where ATF4 knockout caused impaired tumor growth, suggesting that nutrient stress in tumors is not sufficiently strong to activate the pro-apoptotic effects of ATF4 observed in glutamine-starved cell culture conditions. Thus in vitro models replace do not always correlate with in vivo microenvironmental stresses.

ATF4 activity can also contribute to amino acid homeostasis in the tumor microenvironment via metabolic effects in the stromal cells. In particular, cancer-associated fibroblasts display increased stability of ATF4 due to reduced p62-dependent ubiquitination (p62 levels are commonly reduced in cancer-associated fibroblasts compared with normal fibroblasts) [95]. The associated metabolic reprogramming in these cells provides a de novo source of glucose-derived asparagine that can support cancer cell growth, particularly during episodes of glutamine deprivation.

Role of ATF4 in Cell Invasion and Metastasis

Hypoxic activation of the PERK-eIF2α-ATF4 pathway in tumors has been linked to an increased risk of metastasis [96]. ATF4 can stimulate metastasis by promoting cellular tolerance to hypoxia, as discussed earlier, and by enhancing the metastatic properties of cancer cells. Hypoxia is a recognized enhancer of metastasis [97]. Hypoxia promotes numerous steps in the metastatic cascade; remodeling of the extracellular matrix (ECM), intravasation and extravasation of cancer cells, and by promoting a less differentiated cellular state with enhanced motility and invasive properties. Many of these biological changes depend on HIF-1 transcriptional activity, yet ATF4 also contributes in distinct roles. For example, when detached from the ECM, cells experience increased levels of oxidative stress, predisposing them to apoptosis (anoikis). During this process, ATF4 expression is increased and it supports cell survival by transcriptionally up-regulating autophagy via *ATG5*, *ATG7*, and *ULK1* [65]. ATF4, in co-operation with NRF2, induced the transcription of cellular antioxidant responses, particularly the antioxidant enzyme hemeoxygenase 1 (*HO-1*). HO-1 acts to reduce ROS levels following matrix detachment and prevent anoikis. Importantly, these steps are necessary for colonization of cells in the lungs of mice, demonstrating that ATF4 is required for metastasis in this context [65]. PERK has also been shown to promote survival following ECM detachment by inducing autophagy through LKB1-AMPK signaling [98]. Consistently, circulating tumor cells in the bloodstream have increased ATF4 expression [99].

The epithelial-mesenchymal transition (EMT) is a process of transcriptional reprogramming that causes carcinoma cells to reversibly shift into a less differentiated state [100]. During EMT, cells lose their epithelial characteristics including cell-cell adhesion and cellular polarity and acquire mesenchymal attributes including motility, invasiveness, and stem cell-like characteristics. These changes in phenotype promote cancer cell dissemination and metastasis [101, 102]. Tumor hypoxia stimulates cells to undergo EMT, and HIF-1 participates in this process [103]. During EMT, cells increase the expression of secreted ECM proteins, and this results in an increase in EnR stress and a reliance on PERK-eIF2α-ATF4 signaling for maintaining proteostasis and cell survival [104]. Exacerbating this EMT-induced stress using pharmacological EnR stressors including tunicamycin and thapsigargin results in cell death. Similarly, cells that have undergone EMT display increased sensitivity to PERK inhibition, further demonstrating that PERK-eIF2α-ATF4 signaling is required to sustain EMT biology [104].

Independent reports showed that knockdown of ATF4 prevented EMT in gastric cancer cells exposed to severe hypoxia [105]. ATF4 knockdown reduced cell migration, invasion, and metastasis, whereas ATF4 overexpression enhanced these processes [106]. Consistent with these findings, analysis of clinical transcriptomic datasets demonstrated that EMT and ATF4 gene signatures were strongly correlated in their expression across several tumor types including breast, colon, gastric, and lung cancer [104].

ATF4 has also been implicated in non-canonical (β-catenin-independent) Wnt signaling [107]. In this report, non-canonical Wnt ligands (Wnt5a/b) signal via ROR2, DVL2, ATF2, and ATF4 to promote proliferation of colon cancer cells in a β-catenin-independent manner.

Metastasis depends on remodeling of the ECM. ATF4 can stimulate ECM remodeling by transcriptionally up-regulating the matrix degrading enzymes MMP2 and MMP7 [106]. ATF4 also promotes metastasis by transcriptionally up-regulating lysosomal associated membrane protein 3 (*LAMP3*) [96]. LAMP3 is required for cell migration and invasion during hypoxia and for metastasis in animal models although the exact mechanism has not been fully elucidated [108–110].

Recently, co-recruitment of ATF4 with steroid receptor coactivator-3 (SRC-3) was demonstrated in highly glycolytic breast cancers [111]. In this study, the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) was shown to phosphorylate SRC-3 at serine 857 resulting in increased transcriptional activity. Activated SRC-3 supported purine biosynthesis via transcriptional up-regulation of transketolase (*TKT*), adenosine monophosphate deaminase-1 (*AMPD1*), and xanthine dehydrogenase (*XDH*). This transcriptional program and recruitment of SRC-3 to gene promoters was dependent on direct interaction with ATF4, in conditions that favored elevated glycolysis (high glucose, PFKFB4 expression). SRC-3 expression promoted both the growth and metastatic dissemination of breast tumor xenograft models. Furthermore, phosphorylated SRC-3 was associated with worse survival outcomes in breast cancer patients. Thus, the metabolic re-orchestration mediated by ATF4 is co-opted in aggressive cancers and results in poorer outcome.

These studies highlight the important role that ATF4 biology plays at multiple stages of the metastatic cascade including maintenance of the EMT phenotype, resistance to anoikis, and promotion of ECM remodeling.

ATF4 Promotes Angiogenesis

Cells respond to hypoxia by secreting pro-angiogenic factors in an attempt to increase tissue oxygenation. HIF-1 plays a particularly important role in this response. In addition, several studies have demonstrated that the PERK-eIF2α-ATF4 pathway also contributes to tumor angiogenesis [112]. For example, tumors comprised of PERK-deficient cells grow slower than control tumors and have reduced microvessel density [113]. Similarly, potent and selective ATP-competitive small molecule inhibitors of PERK also display anti-angiogenic effects in tumor xenograft models, supporting the notion that PERK-eIF2α-ATF4 signaling is pro-angiogenic in tumors [114].

These observations are supported by the demonstration that ATF4 promotes transcriptional up-regulation of several pro-angiogenic factors during episodes of glucose deprivation [115]. Glucose deprivation or treatment with pharmacological EnR stressors activated the UPR resulting in PERK- and ATF4-dependent induction of *VEGFA*, *FGF2,* and *IL6*. ChIP studies confirmed ATF4 binding at the *VEGFA* gene promoter region. Amino acid deprivation also results in up-regulation of VEGFA secretion [116]. In this situation, both GCN2 and ATF4 are required for *VEGFA* induction. Tumor xenografts consisting of GCN2-deficient cancer cells grow with slower kinetics and have reduced microvessel density compared with controls [116]. Consistently, ATF4 was required for hypoxic transcription of *VEGFA* in osteoblasts. However, in this situation ATF4 was demonstrated to act by promoting the stability of HIF-1α [117]. This interesting finding highlights the potential for crosstalk between transcriptional responses during hypoxia.

ATF4 overexpression has been demonstrated to promote tumor growth by increasing microvessel density [118]. In this study, the effect was due to recruitment of pro-angiogenic macrophages to the tumor via ATF4-dependent secretion of macrophage colony stimulating factor (M-CSF). However, in contrast to previous findings, Liu et al. did not observe ATF4-dependent transcription of *VEGFA*, highlighting the potential for cell-type specific differences in these pathways. A recent report highlighted the importance of ATF4 activity in the disease progression of endometrial cancer [119]. In this work, the transcriptional up-regulation of *CCL2* by ATF4 was responsible for stimulation of tumor growth by increasing tumor recruitment and infiltration of macrophages.

GCN2-eIF2α-ATF4 signaling also promotes angiogenesis in conditions of sulfur amino acid (methionine and cysteine) deprivation [120]. Dietary restriction of sulfur amino acids results in GCN2/ATF4-dependent angiogenesis in mouse skeletal muscle. In this context, ATF4 is required for transcriptional up-regulation of cystathionine-γ-lyase (in addition to induction of VEGFA), leading to increased production of H_2S. H_2S acts by repressing mitochondrial electron transport and oxidative phosphorylation in endothelial cells resulting in activation of AMPK signaling and increased glucose uptake and glycolytic ATP production. These pro-angiogenic effects occur independently of hypoxia or HIF-1α.

Thus, ATF4 directs diverse biological functions that support several pro-angiogenic mechanisms.

ATF4 Activity in Tumor Infiltrating Immune Cells

While ATF4 confers pro-survival characteristics to cancer cells allowing them to resist stresses of the tumor microenvironment, this same activity can provide metabolic resilience to immune cells, possibly allowing for stronger antitumor T cell responses. For example, ATF4 expression is increased in $CD4^+$ T cells in response to oxidizing environments and amino acid deprivation [121]. ATF4 stimulates amino acid synthesis and uptake to restore mTORC1 activity, drives metabolic reprogramming to induce glycolysis, glutaminolysis, and oxidative phosphorylation, and provides nutrients for both anabolic and energetic needs. Ultimately, ATF4 activity is required for proper differentiation of T helper cell (Th) subsets. The implications of this finding on tumor biology have not yet been widely investigated. However, it is likely that ATF4 activity has key roles in maturation and resilience of subsets of antitumor immune cells, and these roles need to be considered in the development of ATF4-targeted therapeutics.

Intercellular Transmission of the Unfolded Protein Response

Cells experiencing elevated levels of EnR stress have been found to secrete signals that result in increased UPR signaling in neighboring cells [122]. This transmissible form of EnR stress, termed TERS, and the increased PERK-ATF4 signal in recipient cells, resulted in improved cellular fitness and impaired responsiveness to either proteasome inhibition or taxane treatment. This newly appreciated phenomenon provides an additional mechanism that may "prime" cancer cells within the tumor with adaptive pro-survival properties before they experience episodes of microenvironmental or pharmacologic stress.

Role of ATF4 in Therapy Resistance

ATF4 Activation in Resistance to Chemotherapy

Acquired drug resistance is a major reason for failure of chemotherapeutic agents. ATF4 has been implicated in cellular resistance to several anticancer agents including cisplatin, doxorubicin, etoposide, gemcitabine, SN-38, and vincristine [80, 123, 124]. Several reports have demonstrated that ATF4 knockdown sensitized cells to these agents, whereas ATF4 overexpression conferred drug resistance [80, 125]. This multidrug resistance phenotype has been attributed to drug efflux via ATF4-dependent transcription of ATP-binding cassette (ABC) membrane transporters ABCC2 and ABCG2 (Fig. 4.3a) [80]. ATF4-dependent transcription of STAT3 (and its target genes BCL2, Survivin, and MRP1) caused a multidrug resistance

phenotype in esophageal cancer cells [125]. Furthermore, ATF4-dependent GSH biosynthesis enhanced drug resistance, and this was abolished by an inhibitor of GSH biosynthesis (Fig. 4.3b) [80, 126]. Up-regulation of sirtuin 1 (SIRT1) has also been reported as an ATF4-induced mechanism of multidrug resistance [127]. SIRT1 plays multiple roles in drug resistance including inhibition of pro-apoptotic responses, promotion of DNA damage repair, and acquisition of cancer stem cell properties [128]. Recent work also demonstrated a role for ATF4 activity in conferring resistance to cell death by ferroptosis, via up-regulation of the xCT glutamate/cystine antiporter subunit *SLC7A11* [112].

ATF4 is induced in response to certain drugs, where it acts as a resistance mechanism. For example, proteasome inhibition by Bortezomib causes an accumulation of misfolded proteins in the EnR. These proteins can be degraded by autophagy to support restoration of EnR homeostasis (Fig. 4.3c). ATF4 acts to transcriptionally up-regulate LC3B and enhance autophagy during Bortezomib treatment [129]. Knockdown of ATF4 suppressed this activation of autophagy and sensitized breast cancer cells to Bortezomib, highlighting an important role for ATF4 in cellular resistance to proteasome inhibitors. ATF4 also promotes cyto-protective autophagy in head and neck cancer cells treated with the next generation proteasome inhibitors, Carfilzomib and Oprozomib [130] and *MYCN*-amplified neuroblastoma cells treated with the GLI1/2 inhibitor, GANT-61 [131]. Import of cystine via ATF4-dependent up-regulation of xCT has also been implicated in cellular resistance to proteasome inhibition [78].

In addition to killing cells, many chemotherapeutic agents also cause therapy-induced senescence (TIS) [132]. TIS can have beneficial effects including inhibition of tumor growth. However, retention of senescent cells within tumors can have adverse effects if these cells acquire a senescence-associated secretory phenotype that results in the increased secretion of pro-inflammatory cytokines. This change in phenotype to a highly secretory state places an increased load on the EnR resulting in reliance on ATF4 and CHOP and increased dependence on autophagy to maintain proteostasis (Fig. 4.3d) [133]. This work highlights the potential utility of targeting PERK-eIF2α-ATF4 signaling to eliminate or modify TIS secretory biology and improve the efficacy of conventional chemotherapeutic drugs.

A link between sorafenib pharmacology and ATF4 biology was recently uncovered [134]. In acute myeloid leukemia (AML) with an internal tandem duplication (ITD) in Fms-related tyrosine kinase 3 (FLT3) sorafenib treatment resulting in inhibition of FLT3 signaling causes downregulation of ATF4. Reduction in ATF4 levels de-represses the negative regulation of interferon regulatory factor 7 (IRF7) leading to elevated transcription and production of IL-15. The increased IL-15 production by FLT3-ITD AML cells generated metabolically capable leukemia-reactive $CD8^+$ T cells. This finding has significant clinical implications for the treatment of AML using allogeneic hematopoietic cell transplantation, highlighting the utility of sorafenib, and negative impact of ATF4, in this setting.

ATF4 also contributes to cellular resistance to ferroptosis, a recently described iron-dependent mechanism of cell killing, with significant potential to be exploited

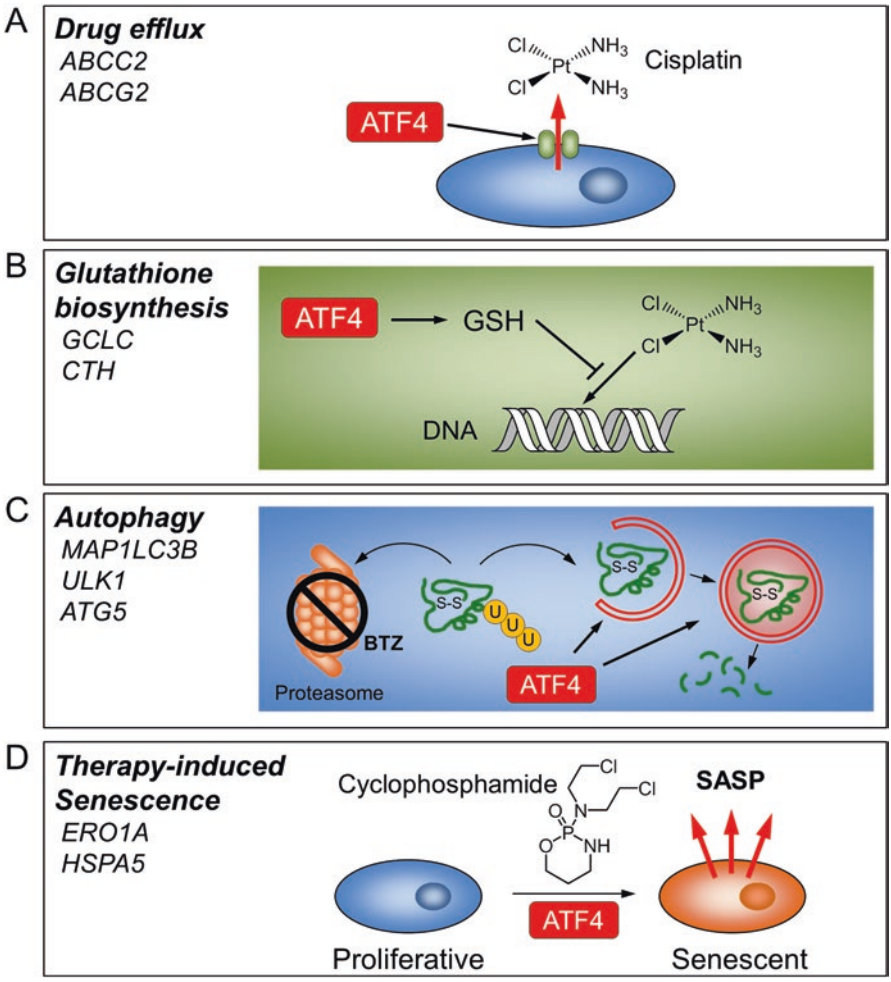

Fig. 4.3 Mechanisms of ATF4-dependent drug resistance. (**a**) ATF4 transcriptionally up-regulates drug efflux transporters including *ABCC2* and *ABCG2*. (**b**) ATF4 transcribes genes that control glutathione biosynthesis including *GCLC* and *CTH*. Glutathione can inactivate cisplatin by producing cisplatin-thiol conjugates, antagonizing its cytotoxic properties. (**c**) ATF4-dependent induction of autophagy can degrade toxic protein aggregates that occur during proteasomal inhibition with Bortezomib (BTZ) to promote drug resistance. (**d**) Treatment with cytotoxic chemotherapeutic agents, including cyclophosphamide, can result in cellular senescence leading to a senescence-associated secretory phenotype (SASP). Acquisition of this state places increased load on the EnR and ATF4 is required to maintain EnR homeostasis during this stress

for clinical benefit [135]. In addition to promoting glutathione synthesis, ATF4 has also been demonstrated to promote GPX4 stability via transcriptional activation of GRP78 [136]. This resistance to ferroptosis mediated by GRP78–GPX4 interactions reduced the sensitivity of pancreatic cancer cells to gemcitabine.

ATF4 Activation in Sensitivity to Chemotherapy

Although ATF4 acts in a pro-survival manner in some situations, it can also contribute to cell death especially when metabolic and EnR stress are excessive or prolonged (as described earlier). ATF4 activity has also been demonstrated as a requirement for drug responsiveness. Notable examples include apoptosis induced by the BRAF-inhibitor vemurafenib [137], combination treatment with BRAF/MEK inhibitor in *NRAS* mutant malignant melanoma [138], apoptosis induction by the NEDD8-activating enzyme inhibitor, MLN4924 [139], p53-independent killing by ONC201 [140, 141], and transcriptional activation of the pro-apoptotic BCL-2 family protein, *NOXA*, in response to cisplatin treatment in p53-null head and neck squamous cell carcinoma cells [142]. ATF4 induction using salubrinal, a selective eIF2α phosphatase inhibitor, also resulted in NOXA up-regulation that sensitized glioma cell lines to temozolomide. Yet, conflicting reports highlight a role for ATF4 in glioma resistance to temozolomide, largely due to transcriptional control of xCT [143].

In some cases, the pro-apoptotic increase in cytosolic Ca^{2+} is a likely trigger of EnR stress, presumably due to depletion of EnR Ca^{2+} stores [137]. Knockdown of ATF4 in this scenario modestly *reduced* the induction of apoptosis, implying that the PERK-eIF2α-ATF4 pathway plays a pro-apoptotic role in drug response. Leukemic cells treated with another RAF inhibitor, sorafenib, also experience increased cytoplasmic Ca^{2+} and induction of EnR stress resulting in activation of all three branches of the UPR [144]. However, in this context, disruption of PERK activity or inhibition of eIF2α phosphorylation *increased* sorafenib-mediated lethality, suggesting that the PERK-eIF2α-ATF4 pathway was anti-apoptotic. Notably, in both reports the observed effects were independent of MEK inhibition. These findings implicate EnR stress in the response to RAF inhibition, but present conflicting outcomes with respect to whether the UPR is pro- or anti-apoptotic.

PERK-eIF2α Signaling in Resistance to Radiotherapy

Hypoxic cells are resistant to killing by radiation [145, 146]. This occurs because molecular oxygen is required to fix DNA free radicals produced by radiation to generate DNA damage and cytotoxicity. Hypoxic cells that survive radiotherapy (RT) can re-populate the tumor and compromise the efficacy of treatment. Selective targeting of hypoxic cells is an effective strategy to overcome this problem [147, 148].

Studies have investigated the potential of targeting molecular changes in hypoxic cells to enhance the response to radiation therapy. Cellular signaling that depends on phosphorylation of eIF2α can be inhibited by expression of a c-terminal fragment of GADD34 (GADD34c) or by a dominant negative eIF2α mutant (S51A). These models have been used to compare the radiation responsiveness of phospho-

eIF2α signaling defective vs. HIF-1-deficient tumor xenografts (shHIF-1α) [149]. Tumors consisting of either phospho-eIF2α signaling defective or HIF-1-deficient cell types had approximately half the number of viable hypoxic cells, confirming that both pathways are important for maintaining hypoxic cells in tumors. Radiation treatment caused a significantly longer growth delay in tumors with phospho-eIF2α signaling defects compared with control tumors. In contrast, knockdown of HIF-1 prior to and during RT (*induced 4 days before to 3 days after initiation of RT*) did not increase the tumor radiosensitivity. This suggests that although both phospho-eIF2α and HIF-1 support hypoxia tolerance in tumors, only phospho-eIF2α is required for maintenance of the radiotherapy-resistant hypoxic subpopulation of cells. Although HIF-1 is not required for cellular tolerance to radiation per se, it is important for tumor regrowth after irradiation, presumably by acting to induce vasculogenesis [149–151]. These findings highlight important differences in the hypoxia tolerance phenotypes mediated by phospho-eIF2α and HIF-1.

Targeting ATF4

Targeting transcription factors with small molecules is challenging due to the large protein–protein and protein–DNA interactions that are implicated in transcription factor activity, although recent successes support the utility of this strategy, for example, HIF-2α-targeting using PT2385 [152]. Rather than targeting ATF4 directly, an alternative approach is either to reduce ATF4 translation by inhibiting upstream eIF2α kinases, or to target phospho-eIF2α signaling itself (Fig. 4.4).

Targeting eIF2α Phosphorylation-Dependent Signaling

A large cell-based screening effort resulted in the discovery of ISRIB, an inhibitor of eIF2α phosphorylation-dependent signaling [153]. ISRIB is a symmetric bis-glycolamide small molecule that inhibits ATF4 activity by interfering with signaling downstream of eIF2α phosphorylation (Fig. 4.4) [153, 154]. ISRIB prevented endogenous ATF4 expression following EnR stress but did not inhibit PERK activation (autophosphorylation) or the IRE1α-XBP1 and ATF6 branches of the UPR. Consequently, the transcriptional up-regulation of ATF4 target genes, *DDIT3* and *GADD34*, was prevented in ISRIB-treated cells. Treatment of cells with ISRIB alone had minimal effect on cell viability. However, ISRIB increased cell death when combined with EnR stress compared with cells treated with EnR stress alone.

The molecular mechanism of action for ISRIB was recently elucidated [155, 156]. These reports describe the structural basis for ISRIB binding to eIF2B, which prevents translation repression and ATF4 signaling in response to eIF2α phosphorylation. ISRIB represents a promising new small molecule for blocking ATF4 expression and further studies investigating its antitumor activity are warranted.

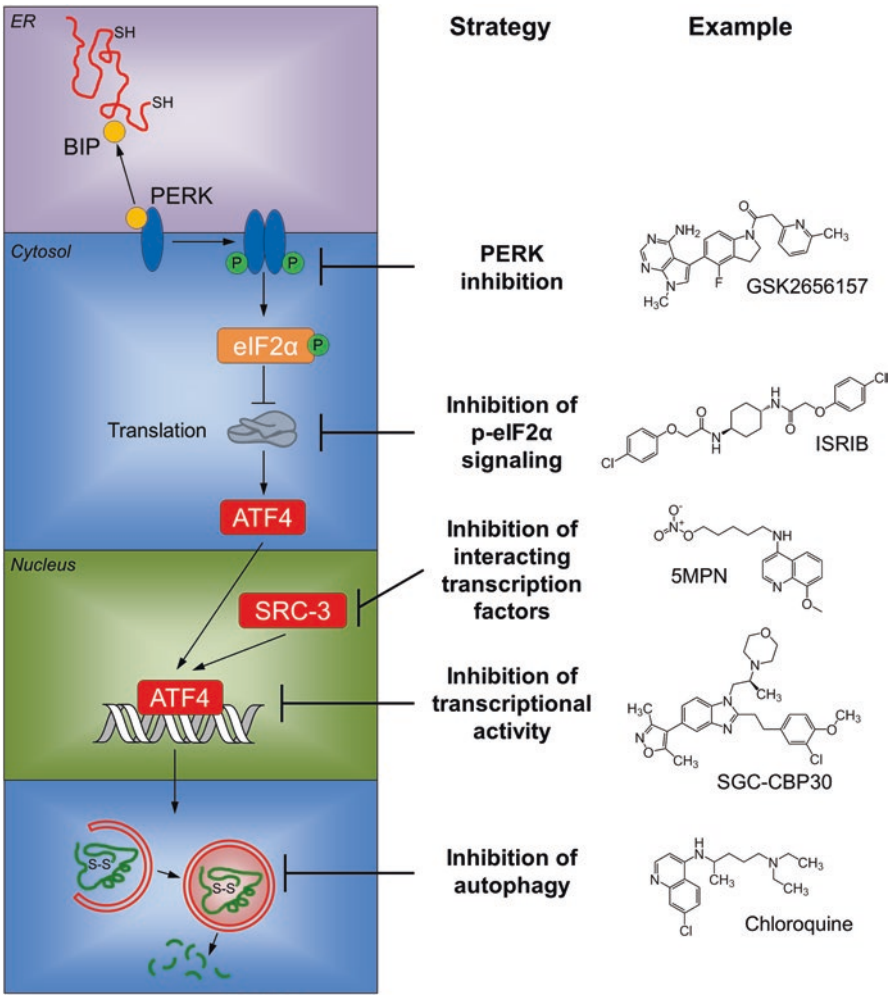

Fig. 4.4 Stages of the PERK-eIF2α-ATF4 pathway that can be targeted with drugs. Inhibition of PERK kinase activity with ATP-competitive inhibitors, e.g., GSK2656157. Inhibition of phospho-eIF2α signaling to ATF4 can be achieved using ISRIB. Interfering with the activation of interacting transcription factors, e.g., SRC-3 using 5MPN to inhibit PFKFB4 activity. Inhibition of ATF4 transcriptional activity may be possible by targeting p300 although this is yet to be demonstrated. Inhibition of downstream processes that depend on ATF4-dependent transcription, e.g., autophagy, is an alternative strategy to target hypoxic ATF4-expressing cancer cells

Targeting PERK

A potent and highly selective PERK inhibitor (GSK2606414) was developed by GlaxoSmithKline [157]. Further optimization of this series resulted in GSK2656157, an orally active ATP-competitive inhibitor of PERK with an IC_{50} of 0.9 nM [114]

(Fig. 4.4). In cells, GSK2656157 inhibited EnR stress-induced phosphorylation of eIF2α and prevented ATF4 expression at concentrations of 10–30 nM. Consequently, the transcriptional up-regulation of ATF4 target genes (*DDIT3*, *HERPUD1*, and *DNAJB9*) was reduced by GSK2656157 in cells treated with tunicamycin to induce EnR stress. GSK2656157 reduced growth of three pancreatic xenograft models and one myeloma xenograft model confirming the anticancer potential of small molecule inhibitors of this pathway. Dynamic contrast-enhanced MRI imaging of tumor xenografts demonstrated that GSK2656157 caused a reduction in vascular perfusion. Furthermore, immunohistochemistry of treated tumors confirmed reduced blood vessel density, emphasizing the importance of PERK in promoting tumor angiogenesis. However, GSK2656157 caused reversible dose-dependent on-target pancreatic toxicity in mice, consistent with previous findings from studies using an inducible PERK knockout system in adult mice [158]. These findings highlight the importance of PERK in pancreatic physiology and emphasize the caution required with clinical development of PERK-targeted therapeutics.

Studies using GSK2606414 demonstrated that pharmacologic inhibition of PERK can sensitize cells to severe or moderate hypoxia (0.2% O_2) [159]. Consistently, cells exposed to PERK inhibitor were highly sensitized to thapsigargin-induced EnR stress. In contrast, a small molecule inhibitor of IRE1α did not reduce cellular tolerance to hypoxia, despite effectively inhibiting IRE1α-dependent splicing of XBP-1. These findings suggest that PERK inhibitors represent a unique approach for preventing UPR-dependent hypoxia tolerance. Further work is needed to define why IRE1α inhibition failed to sensitize cells to severe hypoxia, while shRNA-mediated knockdown of IRE1α did. A second class of orally active PERK inhibitors has recently been reported, providing an independent chemical class to confirm these findings [160].

Although IRE1/XBP1 inhibition was unable to prevent hypoxia tolerance, recent studies have highlighted important opportunities for using IRE1/XBP1-targeted therapies in cancer treatment [161]. In particular, IRE1-XBP1 signaling was found to be induced by MYC in MYC-hyperactivated breast cancers [162]. In this work, pharmacological targeting of IRE1 RNase activity, using the small molecule inhibitor 8866, impaired tumor growth, and enhanced tumor response to docetaxel. Further studies in Triple receptor Negative Breast Cancer (TNBC) cells showed that IRE1 inhibition was effective in reducing the secretion of pro-inflammatory cytokines (particularly IL-6, IL-8, and CXCL1), even after their induction by paclitaxel [163].

A potential mechanism that may permit tumor resistance to eIF2α kinase inhibitors has been proposed [164]. GCN2 deficiency reduces growth of tumor xenografts [81], however, loss of GCN2 in an autochthonous tumor model had no effect on tumor growth [164]. Further investigation revealed that these GCN2-deficient tumors activated PERK as a compensatory mechanism to maintain up-regulation of ATF4, apparently in an eIF2α phosphorylation-independent manner, although this finding needs further clarification. This work highlights the potential for compensatory signaling by other eIF2α kinases when a single eIF2α kinase is selectively

inhibited. Studies to determine whether similar compensatory mechanisms occur in response to PERK pharmacological inhibition are warranted.

Treatment of EnR-stressed cells with an IRE1-XBP1 inhibitor resulted in reduced PERK-ATF4 signaling via a reduction in total eIF2α levels [165]. Notably, the degradation in eIF2α observed relied on PERK-dependent promotion of autophagy. This work highlights how layers of crosstalk between the UPR pathways can be exploited through selective inhibition of individual branches of the UPR to modify the activity of the other branches.

Targeting ATF4 Transcriptional Activity

Targeting post-translational modifications that are required for transcription factor function represents another promising strategy to modulate transcriptional activity. ATF4 is modified by phosphorylation, ubiquitination, and acetylation events. Details of ways to target these changes are reported elsewhere [59].

Recently, much focus has turned to the role of chromatin organization in the regulation of transcription factor activity and definition of cell type identity [166]. This interest in epigenetic control has stimulated the development of potent and selective small molecule inhibitors of epigenetic writers, erasers, and reader proteins [167]. Of interest is the transcriptional co-activator, p300 which is reported to promote ATF4 transcriptional activity by preventing its degradation [168]. Selective inhibitors of both the p300 bromodomain (SGC-CBP30, I-CBP112) and acetyltransferase domain (A-485) have been developed [169–171]. These compounds may provide an opportunity to antagonize the interaction between ATF4 and p300 leading to ATF4 deacetylation, displace the ATF4-p300 complex from acetylated chromatin and/or prevent the establishment of histone acetylation at ATF4-regulated sites in the epigenome. Each of these outcomes would be expected to reduce the level of ATF4 transcriptional activity (Fig. 4.4).

The newly recognized interaction of ATF4 with (PFKFB4-dependent) phosphorylated SRC-3, highlights another opportunity for therapeutic modulation [111]. In cancer cells, PFKFB4 is an important metabolic orchestrator of glycolytic and pentose phosphate pathway activity. 5-(*n*-(8-methoxy-4-quinolyl)amino) pentyl nitrate (5MPN) was reported as a pharmacological inhibitor of PFKFB4 [172]. Inhibition of PFKFB4 activity may provide a strategy to suppress SRC-3 phosphorylation, destabilize ATF4, and prevent the downstream pro-survival adaptive measures.

In certain conditions, it may be beneficial to increase ATF4 activity to enhance or provoke tumor cell death. This may be a desirable outcome in scenarios where ATF4 has a well-characterized pro-apoptotic role, for example, in V600E mutant BRAF melanoma cells following treatment with vemurafenib [137]. Reducing eIF2α dephosphorylation to sustain ATF4 expression can be achieved using the GADD34 inhibitors Sephin1, guanabenz, and salubrinal [173–175].

Targeting Downstream Processes That Depend on ATF4 Transcriptional Activity

ATF4 promotes adaptation to hypoxia, nutrient stress, and ROS by transcriptionally initiating and sustaining autophagic flux. Therefore, inhibiting autophagy is a promising strategy for targeting cancer cells that depend on ATF4 for survival (Fig. 4.4). Cells treated with chloroquine, an inhibitor of lysosomal acidification that prevents degradation of autophagosomal contents, are sensitized to hypoxic exposure [69]. However, chloroquine and related analogues lack potency and improved small molecule inhibitors of autophagy are needed. Additional strategies to impair the adaptive processes that depend on ATF4 target genes could include targeting of angiogenesis, redox balance, or amino acid metabolism.

Conclusions and Future Directions

Activation of the eIF2α-ATF4 pathway provides cancer cells with a key mechanism to tolerate hypoxia and nutrient stress by supporting metabolic homeostasis particularly via increased GSH biosynthesis, replenishment of amino acid pools, and by promoting high rates of autophagy. Important studies have implicated PERK-eIF2α-ATF4 signaling in hypoxic resistance to radiation, providing a clinical setting for the therapeutic development of PERK inhibitors, providing that concerns with pancreatic toxicity can be overcome. The discovery of an eIF2α phosphorylation-dependent signaling inhibitor (ISRIB) and p300 bromodomain/acetyltransferase inhibitors provide new strategies for inhibiting ATF4 activity in tumors and will be useful tools for preclinical therapeutic investigations of this process.

A particularly interesting finding has been the strong anti-angiogenic effects of PERK inhibitors, which suggests an additional important mechanism of antitumor activity [114]. This effect is consistent with reported roles of ATF4 in promoting angiogenesis by transcriptionally activating pro-angiogenic factors [115]. However, genetic models of phospho-eIF2α signaling inhibition do not always display reductions in vascular density [149]. Further investigation is needed to reconcile these findings and to elucidate the potential for direct anti-endothelial effects on tumor vasculature that may be nutritionally deprived. Similarly, much of our understanding is based on in vitro studies which do not accurately model the microenvironmental stresses in tumors. Follow-up studies need to place more emphasis on in vivo characterization of ATF4 biology.

Recent work has highlighted the influence of post-translational modifications in modulating pro-survival versus pro-apoptotic activity of ATF4 [176]. For example, methylation of ATF4 at arginine residue 239 by PRMT1 appears to confer pro-apoptotic activity [177]. Similarly, an understanding of the epigenetic co-factors required for ATF4 activity and epigenomic specificity have only recently emerged and much is yet to be uncovered in this field [178]. A greater understanding of these

modifications, including phosphorylation, methylation, acetylation, and ubiquitination may provide new therapeutic opportunities to fine-tune ATF4 stability and transcriptional activity in efforts to improve cancer therapy.

Drugs designed to selectively eliminate hypoxic/nutrient-deprived cells that depend on ATF4 have the potential to improve patient survival, especially when combined with multimodality treatment regimens containing cytotoxics, molecular targeted agents, and radiotherapy that preferentially kill non-hypoxic/unstressed cells within the tumor. The recognition that secretory phenotypes acquired during therapy-induced senescence are maintained by PERK-eIF2α-ATF4 signaling suggests that inhibiting this pathway may have synergistic effects with conventional chemotherapeutics, including doxorubicin and cyclophosphamide. In addition, the finding that EMT causes high levels of constitutive EnR stress highlights another setting where PERK-eIF2α-ATF4 inhibitors may be beneficial. Treating tumors with high numbers of cells that have undergone EMT, for example, TNBC cancers of the basal-B subtype, may be particularly beneficial. However, these inhibitors are unlikely to be used on their own and there are many potential combination therapies with a strong rationale. Careful clinical trial design and use of biomarkers is needed to predict target patient populations and to validate clinical activity.

Acknowledgments Rodney and Elaine Davies Cancer Research Fellowship, The New Zealand Breast Cancer Foundation Belinda Scott Fellowship, Lottery Health Research, Auckland Medical Research Foundation, European Commission Metoxia Grant No. 222741, Cancer Research UK, Breast Cancer Research Foundation, Oxford NHS Biomedical Research Centre and the Medical Research Council.

References

1. Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nat Rev Cancer. 2002;2(1):38–47.
2. Vaupel P, Hockel M, Mayer A. Detection and characterization of tumor hypoxia using pO2 histography. Antioxid Redox Signal. 2007;9(8):1221–35.
3. Vaupel P, Mayer A, Hockel M. Tumor hypoxia and malignant progression. Methods Enzymol. 2004;381:335–54.
4. Nordsmark M, Bentzen SM, Rudat V, Brizel D, Lartigau E, Stadler P, Becker A, Adam M, Molls M, Dunst J, Terris DJ, Overgaard J. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol. 2005;77(1):18–24.
5. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. Drug Resist Updat. 2011;14(3):191–201.
6. Dales JP, Garcia S, Meunier-Carpentier S, Andrac-Meyer L, Haddad O, Lavaut MN, Allasia C, Bonnier P, Charpin C. Overexpression of hypoxia-inducible factor HIF-1alpha predicts early relapse in breast cancer: retrospective study in a series of 745 patients. Int J Cancer. 2005;116(5):734–9.
7. Gilkes DM, Semenza GL. Role of hypoxia-inducible factors in breast cancer metastasis. Future Oncol. 2013;9(11):1623–36.
8. Rundqvist H, Johnson RS. Tumour oxygenation: implications for breast cancer prognosis. J Intern Med. 2013;274(2):105–12.

9. Yamamoto Y, Ibusuki M, Okumura Y, Kawasoe T, Kai K, Iyama K, Iwase H. Hypoxia-inducible factor 1alpha is closely linked to an aggressive phenotype in breast cancer. Breast Cancer Res Treat. 2008;110(3):465–75.
10. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol. 1953;26(312):638–48.
11. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer. 1955;9(4):539–49.
12. Brown JM. Evidence for acutely hypoxic cells in mouse tumours, and a possible mechanism of reoxygenation. Br J Radiol. 1979;52(620):650–6.
13. Cairns RA, Kalliomaki T, Hill RP. Acute (cyclic) hypoxia enhances spontaneous metastasis of KHT murine tumors. Cancer Res. 2001;61(24):8903–8.
14. Chaudary N, Hill RP. Hypoxia and metastasis. Clin Cancer Res. 2007;13(7):1947–9.
15. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat Rev Cancer. 2008;8(6):425–37.
16. Bennewith KL, Durand RE. Quantifying transient hypoxia in human tumor xenografts by flow cytometry. Cancer Res. 2004;64(17):6183–9.
17. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer. 2011;11(6):393–410.
18. Guise CP, Mowday AM, Ashoorzadeh A, Yuan R, Lin WH, Wu DH, Smaill JB, Patterson AV, Ding K. Bioreductive prodrugs as cancer therapeutics: targeting tumor hypoxia. Chin J Cancer. 2014;33(2):80–6.
19. Hunter FW, Wouters BG, Wilson WR. Hypoxia-activated prodrugs: paths forward in the era of personalised medicine. Br J Cancer. 2016;114(10):1071–7.
20. Mistry IN, Thomas M, Calder EDD, Conway SJ, Hammond EM. Clinical advances of hypoxia-activated prodrugs in combination with radiation therapy. Int J Radiat Oncol Biol Phys. 2017;98(5):1183–96.
21. Hammond EM, Asselin MC, Forster D, O'Connor JP, Senra JM, Williams KJ. The meaning, measurement and modification of hypoxia in the laboratory and the clinic. Clin Oncol. 2014;26(5):277–88.
22. Denny WA, Wilson WR. Bioreducible mustards: a paradigm for hypoxia-selective prodrugs of diffusible cytotoxins (HPDCs). Cancer Metastasis Rev. 1993;12(2):135–51.
23. Guise CP, Abbattista MR, Tipparaju SR, Lambie NK, Su J, Li D, Wilson WR, Dachs GU, Patterson AV. Diflavin oxidoreductases activate the bioreductive prodrug PR-104A under hypoxia. Mol Pharmacol. 2012;81(1):31–40.
24. Hunter FW, Young RJ, Shalev Z, Vellanki RN, Wang J, Gu Y, Joshi N, Sreebhavan S, Weinreb I, Goldstein DP, Moffat J, Ketela T, Brown KR, Koritzinsky M, Solomon B, Rischin D, Wilson WR, Wouters BG. Identification of P450 oxidoreductase as a major determinant of sensitivity to hypoxia-activated prodrugs. Cancer Res. 2015;75:4211–23.
25. Wang J, Foehrenbacher A, Su J, Patel R, Hay MP, Hicks KO, Wilson WR. The 2-nitroimidazole EF5 is a biomarker for oxidoreductases that activate the bioreductive prodrug CEN-209 under hypoxia. Clin Cancer Res. 2012;18(6):1684–95.
26. Pettersen EO, Ebbesen P, Gieling RG, Williams KJ, Dubois L, Lambin P, Ward C, Meehan J, Kunkler IH, Langdon SP, Ree AH, Flatmark K, Lyng H, Calzada MJ, Peso LD, Landazuri MO, Gorlach A, Flamm H, Kieninger J, Urban G, Weltin A, Singleton DC, Haider S, Buffa FM, Harris AL, Scozzafava A, Supuran CT, Moser I, Jobst G, Busk M, Toustrup K, Overgaard J, Alsner J, Pouyssegur J, Chiche J, Mazure N, Marchiq I, Parks S, Ahmed A, Ashcroft M, Pastorekova S, Cao Y, Rouschop KM, Wouters BG, Koritzinsky M, Mujcic H, Cojocari D. Targeting tumour hypoxia to prevent cancer metastasis. From biology, biosensing and technology to drug development: the METOXIA consortium. J Enzyme Inhib Med Chem. 2015;30(5):689–721.
27. Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci. 2012;33(4):207–14.

28. Martinez-Saez O, Gajate Borau P, Alonso-Gordoa T, Molina-Cerrillo J, Grande E. Targeting HIF-2 alpha in clear cell renal cell carcinoma: a promising therapeutic strategy. Crit Rev Oncol Hematol. 2017;111:117–23.
29. Barker HE, Cox TR, Erler JT. The rationale for targeting the LOX family in cancer. Nat Rev Cancer. 2012;12(8):540–52.
30. Chan DA, Sutphin PD, Nguyen P, Turcotte S, Lai EW, Banh A, Reynolds GE, Chi JT, Wu J, Solow-Cordero DE, Bonnet M, Flanagan JU, Bouley DM, Graves EE, Denny WA, Hay MP, Giaccia AJ. Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. Sci Transl Med. 2011;3(94):94ra70.
31. Hu YL, DeLay M, Jahangiri A, Molinaro AM, Rose SD, Carbonell WS, Aghi MK. Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma. Cancer Res. 2012;72(7):1773–83.
32. Kumar K, Wigfield S, Gee HE, Devlin CM, Singleton D, Li JL, Buffa F, Huffman M, Sinn AL, Silver J, Turley H, Leek R, Harris AL, Ivan M. Dichloroacetate reverses the hypoxic adaptation to bevacizumab and enhances its antitumor effects in mouse xenografts. J Mol Med (Berl). 2013;91(6):749–58.
33. McIntyre A, Patiar S, Wigfield S, Li JL, Ledaki I, Turley H, Leek R, Snell C, Gatter K, Sly WS, Vaughan-Jones RD, Swietach P, Harris AL. Carbonic anhydrase IX promotes tumor growth and necrosis in vivo and inhibition enhances anti-VEGF therapy. Clin Cancer Res. 2012;18(11):3100–11.
34. Singleton DC, Rouhi P, Zois CE, Haider S, Li JL, Kessler BM, Cao Y, Harris AL. Hypoxic regulation of RIOK3 is a major mechanism for cancer cell invasion and metastasis. Oncogene. 2015;34(36):4713–22.
35. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer. 2008;8(11):851–64.
36. Braakman I, Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. Annu Rev Biochem. 2011;80:71–99.
37. Gidalevitz T, Stevens F, Argon Y. Orchestration of secretory protein folding by ER chaperones. Biochim Biophys Acta. 2013;1833(11):2410–24.
38. Creighton TE. Protein folding coupled to disulphide bond formation. Biol Chem. 1997;378(8):731–44.
39. Koritzinsky M, Levitin F, van den Beucken T, Rumantir RA, Harding NJ, Chu KC, Boutros PC, Braakman I, Wouters BG. Two phases of disulfide bond formation have differing requirements for oxygen. J Cell Biol. 2013;203(4):615–27.
40. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334(6059):1081–6.
41. Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. J Cell Biol. 2012;197(7):857–67.
42. Carrara M, Prischi F, Nowak PR, Kopp MC, Ali MM. Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling. elife. 2015b;4:e03522.
43. Carrara M, Prischi F, Nowak PR, Ali MM. Crystal structures reveal transient PERK luminal domain tetramerization in endoplasmic reticulum stress signaling. EMBO J. 2015a;34(11):1589–600.
44. Baird TD, Wek RC. Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. Adv Nutr. 2012;3(3):307–21.
45. Algire MA, Maag D, Lorsch JR. Pi release from eIF2, not GTP hydrolysis, is the step controlled by start-site selection during eukaryotic translation initiation. Mol Cell. 2005;20(2):251–62.
46. Hinnebusch AG, Lorsch JR. The mechanism of eukaryotic translation initiation: new insights and challenges. Cold Spring Harb Perspect Biol. 2012;4(10):a011544.
47. Wek RC, Jiang HY, Anthony TG. Coping with stress: eIF2 kinases and translational control. Biochem Soc Trans. 2006;34(Pt 1):7–11.

48. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell. 2000;6(5):1099–108.
49. Lu PD, Harding HP, Ron D. Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. J Cell Biol. 2004;167(1):27–33.
50. Vattem KM, Wek RC. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc Natl Acad Sci U S A. 2004;101(31):11269–74.
51. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell. 2003;11(3):619–33.
52. Zhou J, Wan J, Shu XE, Mao Y, Liu XM, Yuan X, Zhang X, Hess ME, Bruning JC, Qian SB. N(6)-methyladenosine guides mRNA alternative translation during integrated stress response. Mol Cell. 2018;69(4):636–647 e637.
53. Adjibade P, Grenier St-Sauveur V, Bergeman J, Huot ME, Khandjian EW, Mazroui R. DDX3 regulates endoplasmic reticulum stress-induced ATF4 expression. Sci Rep. 2017;7(1):13832.
54. Park Y, Reyna-Neyra A, Philippe L, Thoreen CC. mTORC1 balances cellular amino acid supply with demand for protein synthesis through post-transcriptional control of ATF4. Cell Rep. 2017;19(6):1083–90.
55. Paolini NA, Moore KS, di Summa FM, Fokkema I, t Hoen PAC, von Lindern M. Ribosome profiling uncovers selective mRNA translation associated with eIF2 phosphorylation in erythroid progenitors. PLoS One. 2018;13(4):e0193790.
56. Lee YY, Cevallos RC, Jan E. An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation. J Biol Chem. 2009;284(11):6661–73.
57. Palam LR, Baird TD, Wek RC. Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. J Biol Chem. 2011;286(13):10939–49.
58. Ameri K, Harris AL. Activating transcription factor 4. Int J Biochem Cell Biol. 2008;40(1):14–21.
59. Singleton DC, Harris AL. Targeting the ATF4 pathway in cancer therapy. Expert Opin Ther Targets. 2012;16(12):1189–202.
60. Blais JD, Filipenko V, Bi M, Harding HP, Ron D, Koumenis C, Wouters BG, Bell JC. Activating transcription factor 4 is translationally regulated by hypoxic stress. Mol Cell Biol. 2004;24(17):7469–82.
61. Rzymski T, Paantjens A, Bod J, Harris AL. Multiple pathways are involved in the anoxia response of SKIP3 including HuR-regulated RNA stability, NF-kappaB and ATF4. Oncogene. 2008;27(33):4532–43.
62. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, Harding H, Novoa I, Varia M, Raleigh J, Scheuner D, Kaufman RJ, Bell J, Ron D, Wouters BG, Koumenis C. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. EMBO J. 2005;24(19):3470–81.
63. Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, Yuan CL, Krokowski D, Wang S, Hatzoglou M, Kilberg MS, Sartor MA, Kaufman RJ. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat Cell Biol. 2013;15(5):481–90.
64. Boya P, Reggiori F, Codogno P. Emerging regulation and functions of autophagy. Nat Cell Biol. 2013;15(7):713–20.
65. Dey S, Sayers CM, Verginadis II, Lehman SL, Cheng Y, Cerniglia GJ, Tuttle SW, Feldman MD, Zhang PJ, Fuchs SY, Diehl JA, Koumenis C. ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis. J Clin Invest. 2015;125(7):2592–608.
66. Rouschop KM, Ramaekers CH, Schaaf MB, Keulers TG, Savelkouls KG, Lambin P, Koritzinsky M, Wouters BG. Autophagy is required during cycling hypoxia to lower production of reactive oxygen species. Radiother Oncol. 2009;92(3):411–6.
67. Koritzinsky M, Wouters BG. The roles of reactive oxygen species and autophagy in mediating the tolerance of tumor cells to cycling hypoxia. Semin Radiat Oncol. 2013;23(4):252–61.

68. Rzymski T, Milani M, Pike L, Buffa F, Mellor HR, Winchester L, Pires I, Hammond E, Ragoussis I, Harris AL. Regulation of autophagy by ATF4 in response to severe hypoxia. Oncogene. 2010;29(31):4424–35.
69. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, Voncken JW, Lambin P, van der Kogel AJ, Koritzinsky M, Wouters BG. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP 1LC3B and ATG5. J Clin Investig. 2010;120(1):127–41.
70. Pike LR, Singleton DC, Buffa F, Abramczyk O, Phadwal K, Li JL, Simon AK, Murray JT, Harris AL. Transcriptional up-regulation of ULK1 by ATF4 contributes to cancer cell survival. Biochem J. 2013;449(2):389–400.
71. B'Chir W, Maurin AC, Carraro V, Averous J, Jousse C, Muranishi Y, Parry L, Stepien G, Fafournoux P, Bruhat A. The eIF2alpha/ATF4 pathway is essential for stress-induced autophagy gene expression. Nucleic Acids Res. 2013;41(16):7683–99.
72. Heydt Q, Larrue C, Saland E, Bertoli S, Sarry JE, Besson A, Manenti S, Joffre C, Mansat-De Mas V. Oncogenic FLT3-ITD supports autophagy via ATF4 in acute myeloid leukemia. Oncogene. 2018;37(6):787–97.
73. Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, Qiu B, Zhang H, Cerniglia G, Bi M, Li Y, Gao Y, Liu H, Li C, Maity A, Thomas-Tikhonenko A, Perl AE, Koong A, Fuchs SY, Diehl JA, Mills IG, Ruggero D, Koumenis C. ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest. 2012;122(12):4621–34.
74. Horiguchi M, Koyanagi S, Okamoto A, Suzuki SO, Matsunaga N, Ohdo S. Stress-regulated transcription factor ATF4 promotes neoplastic transformation by suppressing expression of the INK4a/ARF cell senescence factors. Cancer Res. 2012;72(2):395–401.
75. Qing G, Li B, Vu A, Skuli N, Walton ZE, Liu X, Mayes PA, Wise DR, Thompson CB, Maris JM, Hogarty MD, Simon MC. ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. Cancer Cell. 2012;22(5):631–44.
76. Gorlach A, Dimova EY, Petry A, Martinez-Ruiz A, Hernansanz-Agustin P, Rolo AP, Palmeira CM, Kietzmann T. Reactive oxygen species, nutrition, hypoxia and diseases: problems solved? Redox Biol. 2015;6:372–85.
77. Adams CM. Role of the transcription factor ATF4 in the anabolic actions of insulin and the anti-anabolic actions of glucocorticoids. J Biol Chem. 2007;282(23):16744–53.
78. Ye P, Mimura J, Okada T, Sato H, Liu T, Maruyama A, Ohyama C, Itoh K. Nrf2- and ATF4-dependent upregulation of xCT modulates the sensitivity of T24 bladder carcinoma cells to proteasome inhibition. Mol Cell Biol. 2014;34(18):3421–34.
79. Dickhout JG, Carlisle RE, Jerome DE, Mohammed-Ali Z, Jiang H, Yang G, Mani S, Garg SK, Banerjee R, Kaufman RJ, Maclean KN, Wang R, Austin RC. Integrated stress response modulates cellular redox state via induction of cystathionine gamma-lyase: cross-talk between integrated stress response and thiol metabolism. J Biol Chem. 2012;287(10):7603–14.
80. Igarashi T, Izumi H, Uchiumi T, Nishio K, Arao T, Tanabe M, Uramoto H, Sugio K, Yasumoto K, Sasaguri Y, Wang KY, Otsuji Y, Kohno K. Clock and ATF4 transcription system regulates drug resistance in human cancer cell lines. Oncogene. 2007;26(33):4749–60.
81. Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN, Bobrovnikova-Marjon E, Diehl JA, Ron D, Koumenis C. The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. EMBO J. 2010;29(12):2082–96.
82. Chen MS, Wang SF, Hsu CY, Yin PH, Yeh TS, Lee HC, Tseng LM. CHAC1 degradation of glutathione enhances cystine-starvation-induced necroptosis and ferroptosis in human triple negative breast cancer cells via the GCN2-eIF2alpha-ATF4 pathway. Oncotarget. 2017;8(70):114588–602.
83. Mazor KM, Stipanuk MH. GCN2- and eIF2alpha-phosphorylation-independent, but ATF4-dependent, induction of CARE-containing genes in methionine-deficient cells. Amino Acids. 2016;48(12):2831–42.
84. Timosenko E, Ghadbane H, Silk JD, Shepherd D, Gileadi U, Howson LJ, Laynes R, Zhao Q, Strausberg RL, Olsen LR, Taylor S, Buffa FM, Boyd R, Cerundolo V. Nutritional stress

induced by tryptophan-degrading enzymes results in ATF4-dependent reprogramming of the amino acid transporter profile in tumor cells. Cancer Res. 2016;76(21):6193–204.
85. Ye J, Mancuso A, Tong X, Ward PS, Fan J, Rabinowitz JD, Thompson CB. Pyruvate kinase M2 promotes de novo serine synthesis to sustain mTORC1 activity and cell proliferation. Proc Natl Acad Sci U S A. 2012;109(18):6904–9.
86. Ye J, Palm W, Peng M, King B, Lindsten T, Li MO, Koumenis C, Thompson CB. GCN2 sustains mTORC1 suppression upon amino acid deprivation by inducing Sestrin2. Genes Dev. 2015;29(22):2331–6.
87. Zhang N, Yang X, Yuan F, Zhang L, Wang Y, Wang L, Mao Z, Luo J, Zhang H, Zhu WG, Zhao Y. Increased amino acid uptake supports autophagy-deficient cell survival upon glutamine deprivation. Cell Rep. 2018;23(10):3006–20.
88. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2016;23(1):27–47.
89. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16(10):619–34.
90. Kamphorst JJ, Nofal M, Commisso C, Hackett SR, Lu W, Grabocka E, Vander Heiden MG, Miller G, Drebin JA, Bar-Sagi D, Thompson CB, Rabinowitz JD. Human pancreatic cancer tumors are nutrient poor and tumor cells actively scavenge extracellular protein. Cancer Res. 2015;75(3):544–53.
91. Pan M, Reid MA, Lowman XH, Kulkarni RP, Tran TQ, Liu X, Yang Y, Hernandez-Davies JE, Rosales KK, Li H, Hugo W, Song C, Xu X, Schones DE, Ann DK, Gradinaru V, Lo RS, Locasale JW, Kong M. Regional glutamine deficiency in tumours promotes dedifferentiation through inhibition of histone demethylation. Nat Cell Biol. 2016;18(10):1090–101.
92. Chen R, Zou Y, Mao D, Sun D, Gao G, Shi J, Liu X, Zhu C, Yang M, Ye W, Hao Q, Li R, Yu L. The general amino acid control pathway regulates mTOR and autophagy during serum/glutamine starvation. J Cell Biol. 2014;206(2):173–82.
93. Lampa M, Arlt H, He T, Ospina B, Reeves J, Zhang B, Murtie J, Deng G, Barberis C, Hoffmann D, Cheng H, Pollard J, Winter C, Richon V, Garcia-Escheverria C, Adrian F, Wiederschain D, Srinivasan L. Glutaminase is essential for the growth of triple-negative breast cancer cells with a deregulated glutamine metabolism pathway and its suppression synergizes with mTOR inhibition. PLoS One. 2017;12(9):e0185092.
94. Gwinn DM, Lee AG, Briones-Martin-Del-Campo M, Conn CS, Simpson DR, Scott AI, Le A, Cowan TM, Ruggero D, Sweet-Cordero EA. Oncogenic KRAS regulates amino acid homeostasis and asparagine biosynthesis via ATF4 and alters sensitivity to L-asparaginase. Cancer Cell. 2018;33(1):91–107 e106.
95. Linares JF, Cordes T, Duran A, Reina-Campos M, Valencia T, Ahn CS, Castilla EA, Moscat J, Metallo CM, Diaz-Meco MT. ATF4-induced metabolic reprograming is a synthetic vulnerability of the p62-deficient tumor stroma. Cell Metab. 2017;26(6):817–829 e816.
96. Mujcic H, Hill RP, Koritzinsky M, Wouters BG. Hypoxia signaling and the metastatic phenotype. Curr Mol Med. 2014;14(5):565–79.
97. Rankin EB, Giaccia AJ. Hypoxic control of metastasis. Science. 2016;352(6282):175–80.
98. Avivar-Valderas A, Bobrovnikova-Marjon E, Alan Diehl J, Bardeesy N, Debnath J, Aguirre-Ghiso JA. Regulation of autophagy during ECM detachment is linked to a selective inhibition of mTORC1 by PERK. Oncogene. 2013;32(41):4932–40.
99. Ameri K, Luong R, Zhang H, Powell AA, Montgomery KD, Espinosa I, Bouley DM, Harris AL, Jeffrey SS. Circulating tumour cells demonstrate an altered response to hypoxia and an aggressive phenotype. Br J Cancer. 2010;102(3):561–9.
100. Tam WL, Weinberg RA. The epigenetics of epithelial-mesenchymal plasticity in cancer. Nat Med. 2013;19(11):1438–49.
101. Nieto MA. The ins and outs of the epithelial to mesenchymal transition in health and disease. Annu Rev Cell Dev Biol. 2011;27:347–76.
102. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004;117(7):927–39.

103. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC, Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat Cell Biol. 2008;10(3):295–305.
104. Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q, Gupta PB. Epithelial-to-mesenchymal transition activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress. Cancer Discov. 2014;4(6):702–15.
105. Shen X, Xue Y, Si Y, Wang Q, Wang Z, Yuan J, Zhang X. The unfolded protein response potentiates epithelial-to-mesenchymal transition (EMT) of gastric cancer cells under severe hypoxic conditions. Med Oncol. 2015;32(1):447.
106. Zhu H, Chen X, Chen B, Chen B, Song W, Sun D, Zhao Y. Activating transcription factor 4 promotes esophageal squamous cell carcinoma invasion and metastasis in mice and is associated with poor prognosis in human patients. PLoS One. 2014;9(7):e103882.
107. Voloshanenko O, Schwartz U, Kranz D, Rauscher B, Linnebacher M, Augustin I, Boutros M. β-catenin-independent regulation of Wnt target genes by RoR2 and ATF2/ATF4 in colon cancer cells. Sci Rep. 2018;8(1):3178.
108. Mujcic H, Nagelkerke A, Rouschop KM, Chung S, Chaudary N, Span PN, Clarke B, Milosevic M, Sykes J, Hill RP, Koritzinsky M, Wouters BG. Hypoxic activation of the PERK/eIF2alpha arm of the unfolded protein response promotes metastasis through induction of LAMP3. Clin Cancer Res. 2013;19(22):6126–37.
109. Mujcic H, Rzymski T, Rouschop KM, Koritzinsky M, Milani M, Harris AL, Wouters BG. Hypoxic activation of the unfolded protein response (UPR) induces expression of the metastasis-associated gene LAMP3. Radiother Oncol. 2009;92(3):450–9.
110. Nagelkerke A, Bussink J, Mujcic H, Wouters BG, Lehmann S, Sweep FC, Span PN. Hypoxia stimulates migration of breast cancer cells via the PERK/ATF4/LAMP3-arm of the unfolded protein response. Breast Cancer Res. 2013;15(1):R2.
111. Dasgupta S, Rajapakshe K, Zhu B, Nikolai BC, Yi P, Putluri N, Choi JM, Jung SY, Coarfa C, Westbrook TF, Zhang XH, Foulds CE, Tsai SY, Tsai MJ, O'Malley BW. Metabolic enzyme PFKFB4 activates transcriptional coactivator SRC-3 to drive breast cancer. Nature. 2018;556(7700):249–54.
112. Chen D, Fan Z, Rauh M, Buchfelder M, Eyupoglu IY, Savaskan N. ATF4 promotes angiogenesis and neuronal cell death and confers ferroptosis in a xCT-dependent manner. Oncogene. 2017;36(40):5593–608.
113. Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, Koumenis C, Harding HP, Ron D, Holcik M, Bell JC. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol Cell Biol. 2006;26(24):9517–32.
114. Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, Stanley TB, Sanders B, Goetz A, Gaul N, Choudhry AE, Alsaid H, Jucker BM, Axten JM, Kumar R. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013;73(6):1993–2002.
115. Wang Y, Alam GN, Ning Y, Visioli F, Dong Z, Nor JE, Polverini PJ. The unfolded protein response induces the angiogenic switch in human tumor cells through the PERK/ATF4 pathway. Cancer Res. 2012;72(20):5396–406.
116. Wang Y, Ning Y, Alam GN, Jankowski BM, Dong Z, Nor JE, Polverini PJ. Amino acid deprivation promotes tumor angiogenesis through the GCN2/ATF4 pathway. Neoplasia. 2013;15(8):989–97.
117. Zhu K, Jiao H, Li S, Cao H, Galson DL, Zhao Z, Zhao X, Lai Y, Fan J, Im HJ, Chen D, Xiao G. ATF4 promotes bone angiogenesis by increasing VEGF expression and release in the bone environment. J Bone Miner Res. 2013;28(9):1870–84.
118. Liu C, Li Z, Wang L, Tong L, He N, Chen Y, Liu Y, Wu Z, Sun P, Xiang R, Ren G, Su W. Activating transcription factor 4 promotes angiogenesis of breast cancer through enhanced macrophage recruitment. Biomed Res Int. 2015;2015:974615.
119. Liu B, Chen P, Xi D, Zhu H, Gao Y. ATF4 regulates CCL2 expression to promote endometrial cancer growth by controlling macrophage infiltration. Exp Cell Res. 2017;360(2):105–12.

120. Longchamp A, Mirabella T, Arduini A, MacArthur MR, Das A, Trevino-Villarreal JH, Hine C, Ben-Sahra I, Knudsen NH, Brace LE, Reynolds J, Mejia P, Tao M, Sharma G, Wang R, Corpataux JM, Haefliger JA, Ahn KH, Lee CH, Manning BD, Sinclair DA, Chen CS, Ozaki CK, Mitchell JR. Amino acid restriction triggers angiogenesis via GCN2/ATF4 regulation of VEGF and H2S production. Cell. 2018;173(1):117–129 e114.
121. Yang X, Xia R, Yue C, Zhai W, Du W, Yang Q, Cao H, Chen X, Obando D, Zhu Y, Chen X, Chen JJ, Piganelli J, Wipf P, Jiang Y, Xiao G, Wu C, Jiang J, Lu B. ATF4 regulates CD4(+) T cell immune responses through metabolic reprogramming. Cell Rep. 2018;23(6):1754–66.
122. Rodvold JJ, Chiu KT, Hiramatsu N, Nussbacher JK, Galimberti V, Mahadevan NR, Willert K, Lin JH, Zanetti M. Intercellular transmission of the unfolded protein response promotes survival and drug resistance in cancer cells. Sci Signal. 2017;10(482):eaah7177.
123. Palam LR, Gore J, Craven KE, Wilson JL, Korc M. Integrated stress response is critical for gemcitabine resistance in pancreatic ductal adenocarcinoma. Cell Death Dis. 2015;6:e1913.
124. Tanabe M, Izumi H, Ise T, Higuchi S, Yamori T, Yasumoto K, Kohno K. Activating transcription factor 4 increases the cisplatin resistance of human cancer cell lines. Cancer Res. 2003;63(24):8592–5.
125. Zhu H, Chen X, Chen B, Chen B, Fan J, Song W, Xie Z, Jiang D, Li Q, Zhou M, Sun D, Zhao Y. Activating transcription factor 4 mediates a multidrug resistance phenotype of esophageal squamous cell carcinoma cells through transactivation of STAT3 expression. Cancer Lett. 2014;354(1):142–52.
126. Zhang Z, Yin J, Zhang C, Liang N, Bai N, Chang A, Liu Y, Li Z, Tan X, Li N, Lv D, Xiang R, Tian Y, Liu C. Activating transcription factor 4 increases chemotherapeutics resistance of human hepatocellular carcinoma. Cancer Biol Ther. 2012;13(6):435–42.
127. Zhu H, Xia L, Zhang Y, Wang H, Xu W, Hu H, Wang J, Xin J, Gang Y, Sha S, Xu B, Fan D, Nie Y, Wu K. Activating transcription factor 4 confers a multidrug resistance phenotype to gastric cancer cells through transactivation of SIRT1 expression. PLoS One. 2012;7(2):e31431.
128. Wang Z, Chen W. Emerging roles of SIRT1 in cancer drug resistance. Genes Cancer. 2013;4(3–4):82–90.
129. Milani M, Rzymski T, Mellor HR, Pike L, Bottini A, Generali D, Harris AL. The role of ATF4 stabilization and autophagy in resistance of breast cancer cells treated with Bortezomib. Cancer Res. 2009;69(10):4415–23.
130. Zang Y, Thomas SM, Chan ET, Kirk CJ, Freilino ML, DeLancey HM, Grandis JR, Li C, Johnson DE. Carfilzomib and ONX 0912 inhibit cell survival and tumor growth of head and neck cancer and their activities are enhanced by suppression of Mcl-1 or autophagy. Clin Cancer Res. 2012;18(20):5639–49.
131. Wang J, Huang S, Tian R, Chen J, Gao H, Xie C, Shan Y, Zhang Z, Gu S, Xu M. The protective autophagy activated by GANT-61 in MYCN amplified neuroblastoma cells is mediated by PERK. Oncotarget. 2018;9(18):14413–27.
132. Nardella C, Clohessy JG, Alimonti A, Pandolfi PP. Pro-senescence therapy for cancer treatment. Nat Rev Cancer. 2011;11(7):503–11.
133. Dorr JR, Yu Y, Milanovic M, Beuster G, Zasada C, Dabritz JH, Lisec J, Lenze D, Gerhardt A, Schleicher K, Kratzat S, Purfurst B, Walenta S, Mueller-Klieser W, Graler M, Hummel M, Keller U, Buck AK, Dorken B, Willmitzer L, Reimann M, Kempa S, Lee S, Schmitt CA. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Nature. 2013;501(7467):421–5.
134. Mathew NR, Baumgartner F, Braun L, O'Sullivan D, Thomas S, Waterhouse M, Muller TA, Hanke K, Taromi S, Apostolova P, Illert AL, Melchinger W, Duquesne S, Schmitt-Graeff A, Osswald L, Yan KL, Weber A, Tugues S, Spath S, Pfeifer D, Follo M, Claus R, Lubbert M, Rummelt C, Bertz H, Wasch R, Haag J, Schmidts A, Schultheiss M, Bettinger D, Thimme R, Ullrich E, Tanriver Y, Vuong GL, Arnold R, Hemmati P, Wolf D, Ditschkowski M, Jilg C, Wilhelm K, Leiber C, Gerull S, Halter J, Lengerke C, Pabst T, Schroeder T, Kobbe G, Rosler W, Doostkam S, Meckel S, Stabla K, Metzelder SK, Halbach S, Brummer T, Hu Z, Dengjel J, Hackanson B, Schmid C, Holtick U, Scheid C, Spyridonidis A, Stolzel F, Ordemann R,

Muller LP, Sicre-de-Fontbrune F, Ihorst G, Kuball J, Ehlert JE, Feger D, Wagner EM, Cahn JY, Schnell J, Kuchenbauer F, Bunjes D, Chakraverty R, Richardson S, Gill S, Kroger N, Ayuk F, Vago L, Ciceri F, Muller AM, Kondo T, Teshima T, Klaeger S, Kuster B, Kim DDH, Weisdorf D, van der Velden W, Dorfel D, Bethge W, Hilgendorf I, Hochhaus A, Andrieux G, Borries M, Busch H, Magenau J, Reddy P, Labopin M, Antin JH, Henden AS, Hill GR, Kennedy GA, Bar M, Sarma A, McLornan D, Mufti G, Oran B, Rezvani K, Shah O, Negrin RS, Nagler A, Prinz M, Burchert A, Neubauer A, Beelen D, Mackensen A, von Bubnoff N, Herr W, Becher B, Socie G, Caligiuri MA, Ruggiero E, Bonini C, Hacker G, Duyster J, Finke J, Pearce E, Blazar BR, Zeiser R. Sorafenib promotes graft-versus-leukemia activity in mice and humans through IL-15 production in FLT3-ITD-mutant leukemia cells. Nat Med. 2018;24(3):282–91.

135. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascon S, Hatzios SK, Kagan VE, Noel K, Jiang X, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran Q, Rosenfeld CS, Salnikow K, Tang D, Torti FM, Torti SV, Toyokuni S, Woerpel KA, Zhang DD. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell. 2017;171(2):273–85.
136. Zhu S, Zhang Q, Sun X, Zeh HJ 3rd, Lotze MT, Kang R, Tang D. HSPA5 regulates ferroptotic cell death in cancer cells. Cancer Res. 2017;77(8):2064–77.
137. Beck D, Niessner H, Smalley KS, Flaherty K, Paraiso KH, Busch C, Sinnberg T, Vasseur S, Iovanna JL, Driessen S, Stork B, Wesselborg S, Schaller M, Biedermann T, Bauer J, Lasithiotakis K, Weide B, Eberle J, Schittek B, Schadendorf D, Garbe C, Kulms D, Meier F. Vemurafenib potently induces endoplasmic reticulum stress-mediated apoptosis in BRAFV600E melanoma cells. Sci Signal. 2013;6(260):ra7.
138. Niessner H, Sinnberg T, Kosnopfel C, Smalley KSM, Beck D, Praetorius C, Mai M, Beissert S, Kulms D, Schaller M, Garbe C, Flaherty KT, Westphal D, Wanke I, Meier F. BRAF inhibitors amplify the proapoptotic activity of MEK inhibitors by inducing ER stress in NRAS-mutant melanoma. Clin Cancer Res. 2017;23(20):6203–14.
139. Liu X, Jiang Y, Wu J, Zhang W, Liang Y, Jia L, Yu J, Jeong LS, Li L. NEDD8-activating enzyme inhibitor, MLN4924 (Pevonedistat) induces NOXA-dependent apoptosis through up-regulation of ATF-4. Biochem Biophys Res Commun. 2017b;488(1):1–5.
140. Ishizawa J, Kojima K, Chachad D, Ruvolo P, Ruvolo V, Jacamo RO, Borthakur G, Mu H, Zeng Z, Tabe Y, Allen JE, Wang Z, Ma W, Lee HC, Orlowski R, Sarbassov dos D, Lorenzi PL, Huang X, Neelapu SS, McDonnell T, Miranda RN, Wang M, Kantarjian H, Konopleva M, Davis RE, Andreeff M. ATF4 induction through an atypical integrated stress response to ONC201 triggers p53-independent apoptosis in hematological malignancies. Sci Signal. 2016;9(415):ra17.
141. Kline CL, Van den Heuvel AP, Allen JE, Prabhu VV, Dicker DT, El-Deiry WS. ONC201 kills solid tumor cells by triggering an integrated stress response dependent on ATF4 activation by specific eIF2alpha kinases. Sci Signal. 2016;9(415):ra18.
142. Sharma K, Vu TT, Cook W, Naseri M, Zhan K, Nakajima W, Harada H. p53-independent Noxa induction by cisplatin is regulated by ATF3/ATF4 in head and neck squamous cell carcinoma cells. Mol Oncol. 2018;12(6):788–98.
143. Chen D, Rauh M, Buchfelder M, Eyupoglu IY, Savaskan N. The oxido-metabolic driver ATF4 enhances temozolamide chemo-resistance in human gliomas. Oncotarget. 2017b;8(31):51164–76.
144. Rahmani M, Davis EM, Crabtree TR, Habibi JR, Nguyen TK, Dent P, Grant S. The kinase inhibitor sorafenib induces cell death through a process involving induction of endoplasmic reticulum stress. Mol Cell Biol. 2007;27(15):5499–513.
145. Brown JM. Tumor hypoxia in cancer therapy. Methods Enzymol. 2007;435:297–321.
146. Wardman P. The importance of radiation chemistry to radiation and free radical biology (The 2008 Silvanus Thompson Memorial Lecture). Br J Radiol. 2009;82(974):89–104.
147. Hicks KO, Siim BG, Jaiswal JK, Pruijn FB, Fraser AM, Patel R, Hogg A, Liyanage HD, Dorie MJ, Brown JM, Denny WA, Hay MP, Wilson WR. Pharmacokinetic/pharmacodynamic

modeling identifies SN30000 and SN29751 as tirapazamine analogues with improved tissue penetration and hypoxic cell killing in tumors. Clin Cancer Res. 2010;16(20):4946–57.
148. Peeters SG, Zegers CM, Biemans R, Lieuwes NG, van Stiphout RG, Yaromina A, Sun JD, Hart CP, Windhorst AD, van Elmpt W, Dubois LJ, Lambin P. TH-302 in combination with radiotherapy enhances the therapeutic outcome and is associated with pretreatment [18F] HX4 hypoxia PET imaging. Clin Cancer Res. 2015;21(13):2984–92.
149. Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, van der Kogel AJ, Koritzinsky M, Wouters BG. PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. Proc Natl Acad Sci U S A. 2013;110(12):4622–7.
150. Brown JM. Vasculogenesis: a crucial player in the resistance of solid tumours to radiotherapy. Br J Radiol. 2014;87(1035):20130686.
151. Moeller BJ, Dreher MR, Rabbani ZN, Schroeder T, Cao Y, Li CY, Dewhirst MW. Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity. Cancer Cell. 2005;8(2):99–110.
152. Wallace EM, Rizzi JP, Han G, Wehn PM, Cao Z, Du X, Cheng T, Czerwinski RM, Dixon DD, Goggin BS, Grina JA, Halfmann MM, Maddie MA, Olive SR, Schlachter ST, Tan H, Wang B, Wang K, Xie S, Xu R, Yang H, Josey JA. A small-molecule antagonist of HIF2alpha is efficacious in preclinical models of renal cell carcinoma. Cancer Res. 2016;76(18):5491–500.
153. Sidrauski C, Acosta-Alvear D, Khoutorsky A, Vedantham P, Hearn BR, Li H, Gamache K, Gallagher CM, Ang KK, Wilson C, Okreglak V, Ashkenazi A, Hann B, Nader K, Arkin MR, Renslo AR, Sonenberg N, Walter P. Pharmacological brake-release of mRNA translation enhances cognitive memory. Elife. 2013;2:e00498.
154. Sidrauski C, McGeachy AM, Ingolia NT, Walter P. The small molecule ISRIB reverses the effects of eIF2alpha phosphorylation on translation and stress granule assembly. Elife. 2015;4:e05033.
155. Tsai JC, Miller-Vedam LE, Anand AA, Jaishankar P, Nguyen HC, Renslo AR, Frost A, Walter P. Structure of the nucleotide exchange factor eIF2B reveals mechanism of memory-enhancing molecule. Science. 2018;359(6383):eaaq0939.
156. Zyryanova AF, Weis F, Faille A, Alard AA, Crespillo-Casado A, Sekine Y, Harding HP, Allen F, Parts L, Fromont C, Fischer PM, Warren AJ, Ron D. Binding of ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. Science. 2018;359(6383):1533–6.
157. Axten JM, Romeril SP, Shu A, Ralph J, Medina JR, Feng Y, Li WH, Grant SW, Heerding DA, Minthorn E, Mencken T, Gaul N, Goetz A, Stanley T, Hassell AM, Gampe RT, Atkins C, Kumar R. Discovery of GSK2656157: an optimized PERK inhibitor selected for preclinical development. ACS Med Chem Lett. 2013;4(10):964–8.
158. Gao Y, Sartori DJ, Li C, Yu QC, Kushner JA, Simon MC, Diehl JA. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. Mol Cell Biol. 2012;32(24):5129–39.
159. Cojocari D, Vellanki RN, Sit B, Uehling D, Koritzinsky M, Wouters BG. New small molecule inhibitors of UPR activation demonstrate that PERK, but not IRE1alpha signaling is essential for promoting adaptation and survival to hypoxia. Radiother Oncol. 2013;108(3):541–7.
160. Smith AL, Andrews KL, Beckmann H, Bellon SF, Beltran PJ, Booker S, Chen H, Chung YA, D'Angelo ND, Dao J, Dellamaggiore KR, Jaeckel P, Kendall R, Labitzke K, Long AM, Materna-Reichelt S, Mitchell P, Norman MH, Powers D, Rose M, Shaffer PL, Wu MM, Lipford JR. Discovery of 1H-pyrazol-3(2H)-ones as potent and selective inhibitors of protein kinase R-like endoplasmic reticulum kinase (PERK). J Med Chem. 2015;58(3):1426–41.
161. Rubio-Patino C, Bossowski JP, Chevet E, Ricci JE. Reshaping the immune tumor microenvironment through IRE1 signaling. Trends Mol Med. 2018;24(7):607–14.
162. Zhao N, Cao J, Xu L, Tang Q, Dobrolecki LE, Lv X, Talukdar M, Lu Y, Wang X, Hu DZ, Shi Q, Xiang Y, Wang Y, Liu X, Bu W, Jiang Y, Li M, Gong Y, Sun Z, Ying H, Yuan B, Lin X, Feng XH, Hartig SM, Li F, Shen H, Chen Y, Han L, Zeng Q, Patterson JB, Kaipparettu BA, Putluri N, Sicheri F, Rosen JM, Lewis MT, Chen X. Pharmacological targeting of

MYC-regulated IRE1/XBP1 pathway suppresses MYC-driven breast cancer. J Clin Invest. 2018;128(4):1283–99.
163. Logue SE, McGrath EP, Cleary P, Greene S, Mnich K, Almanza A, Chevet E, Dwyer RM, Oommen A, Legembre P, Godey F, Madden EC, Leuzzi B, Obacz J, Zeng Q, Patterson JB, Jager R, Gorman AM, Samali A. Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. Nat Commun. 2018;9(1):3267.
164. Lehman SL, Ryeom S, Koumenis C. Signaling through alternative Integrated Stress Response pathways compensates for GCN2 loss in a mouse model of soft tissue sarcoma. Sci Rep. 2015;5:11781.
165. Storniolo A, Alfano V, Carbotta S, Ferretti E, Di Renzo L. IRE1alpha deficiency promotes tumor cell death and eIF2alpha degradation through PERK dipendent autophagy. Cell Death Discov. 2018;4:3.
166. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016;17(8):487–500.
167. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M. Epigenetic protein families: a new frontier for drug discovery. Nat Rev Drug Discov. 2012;11(5):384–400.
168. Lassot I, Estrabaud E, Emiliani S, Benkirane M, Benarous R, Margottin-Goguet F. p300 modulates ATF4 stability and transcriptional activity independently of its acetyltransferase domain. J Biol Chem. 2005;280(50):41537–45.
169. Hay DA, Fedorov O, Martin S, Singleton DC, Tallant C, Wells C, Picaud S, Philpott M, Monteiro OP, Rogers CM, Conway SJ, Rooney TP, Tumber A, Yapp C, Filippakopoulos P, Bunnage ME, Muller S, Knapp S, Schofield CJ, Brennan PE. Discovery and optimization of small-molecule ligands for the CBP/p300 bromodomains. J Am Chem Soc. 2014;136(26):9308–19.
170. Lasko LM, Jakob CG, Edalji RP, Qiu W, Montgomery D, Digiammarino EL, Hansen TM, Risi RM, Frey R, Manaves V, Shaw B, Algire M, Hessler P, Lam LT, Uziel T, Faivre E, Ferguson D, Buchanan FG, Martin RL, Torrent M, Chiang GG, Karukurichi K, Langston JW, Weinert BT, Choudhary C, de Vries P, Van Drie JH, McElligott D, Kesicki E, Marmorstein R, Sun C, Cole PA, Rosenberg SH, Michaelides MR, Lai A, Bromberg KD. Discovery of a selective catalytic p300/CBP inhibitor that targets lineage-specific tumours. Nature. 2017;550(7674):128–32.
171. Picaud S, Fedorov O, Thanasopoulou A, Leonards K, Jones K, Meier J, Olzscha H, Monteiro O, Martin S, Philpott M, Tumber A, Filippakopoulos P, Yapp C, Wells C, Che KH, Bannister A, Robson S, Kumar U, Parr N, Lee K, Lugo D, Jeffrey P, Taylor S, Vecellio ML, Bountra C, Brennan PE, O'Mahony A, Velichko S, Muller S, Hay D, Daniels DL, Urh M, La Thangue NB, Kouzarides T, Prinjha R, Schwaller J, Knapp S. Generation of a selective small molecule inhibitor of the CBP/p300 bromodomain for leukemia therapy. Cancer Res. 2015;75(23):5106–19.
172. Chesney J, Clark J, Lanceta L, Trent JO, Telang S. Targeting the sugar metabolism of tumors with a first-in-class 6-phosphofructo-2-kinase (PFKFB4) inhibitor. Oncotarget. 2015;6(20):18001–11.
173. Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D, Yuan J. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science. 2005;307(5711):935–9.
174. Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, Barry N, Sigurdardottir A, Bertolotti A. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. Science. 2015;348(6231):239–42.
175. Tsaytler P, Harding HP, Ron D, Bertolotti A. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. Science. 2011;332(6025):91–4.
176. Wortel IMN, van der Meer LT, Kilberg MS, van Leeuwen FN. Surviving stress: modulation of ATF4-mediated stress responses in normal and malignant cells. Trends Endocrinol Metab. 2017;28(11):794–806.

177. Yuniati L, van der Meer LT, Tijchon E, van Ingen Schenau D, van Emst L, Levers M, Palit SA, Rodenbach C, Poelmans G, Hoogerbrugge PM, Shan J, Kilberg MS, Scheijen B, van Leeuwen FN. Tumor suppressor BTG1 promotes PRMT1-mediated ATF4 function in response to cellular stress. Oncotarget. 2016;7(3):3128–43.
178. Zhao E, Ding J, Xia Y, Liu M, Ye B, Choi JH, Yan C, Dong Z, Huang S, Zha Y, Yang L, Cui H, Ding HF. KDM4C and ATF4 cooperate in transcriptional control of amino acid metabolism. Cell Rep. 2016;14(3):506–19.

Chapter 5
Role of Protein Translation in Unfolded Protein Response

Surojeet Sengupta, V. Craig Jordan, and Robert Clarke

Abstract The unfolded protein response (UPR) is an adaptive mechanism to maintain protein homeostasis by decreasing the accumulation of unfolded proteins in the endoplasmic reticulum (EnR) of cells. EnR stress activates three distinct sensors, namely, inositol requiring protein 1 alpha (IRE1-α), activating transcription factor 6 (ATF6), and protein kinase RNA-like endoplasmic reticulum kinase (PERK), that collectively mitigate the damaging effects of EnR stress. The downstream signaling from the PERK sensor phosphorylates the eukaryotic translational initiation factor 2 alpha (eIF2α) complex that inhibits global protein translation to restore proteostasis and promote cell survival. However, chronic and unmitigated activation of the PERK pathway leads to apoptosis. Phosphorylation of eIF2α is tightly controlled by the two specific regulatory subunits of protein phosphatase 1 (PP1) complex, (1) growth arrest and DNA damage inducible-34 (GADD34) and (2) constitutive repressor of eIF2α phosphorylation (CReP), that are responsible for dephosphorylation of eIF2α. Phospho-eIF2α also directs preferential translational of stress-related genes such as ATF4 and CHOP. This chapter describes the mechanism by which the PERK pathway regulates the protein translational machinery that plays a critical role in deciding cell fate following endoplasmic reticulum stress.

Keywords ATF4 · ATF6 · CHOP · CReP · Endoplasmic reticulum stress · eIF2α · FoxO1 · GADD34 · IRE1α · Metabolism · Neurodegenerative · PERK · uORF

S. Sengupta (✉)
Department of Oncology, Georgetown Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA
e-mail: ss972@georgetown.edu

R. Clarke
Department of Oncology, Georgetown University Medical Center, Washington, DC, USA
e-mail: clarker@georgetown.edu

V. C. Jordan
Department of Breast Medical Oncology, University of Texas MD Anderson Cancer Center, Unit 1354, Houston, TX, USA
e-mail: VCJordan@mdanderson.org

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_5

Introduction

Protein synthesis is a fundamental mechanism in living organism that translates the information encoded in the mRNA molecule. Protein translation in eukaryotes is an extremely complex, energy consuming, multi-tiered process requiring multiple components and thus it is tightly regulated at many levels [1–3]. The endoplasmic reticulum (EnR) is a key cell organelle where protein is synthesized, folded, and achieves structural maturity [4, 5]. Maintenance of protein homeostasis, also known as 'proteostasis', encompasses a concerted interconnecting network of cellular processes that controls the structural, spatial, and functional integrity of the proteins making up the proteome [6]. The regulatory controls for protein synthesis often operate at the levels of transcription and/or translation. Revolutionary technologies in genomics and proteomics have enabled us to develop a comprehensive understanding of gene regulation at the system-level. Recent studies [7] have revealed that levels of mRNA transcripts are often not sufficient to predict the levels of their translated protein products. Instead, ribosome occupancy serves as a more reliable predictor of protein levels than levels of their mRNA. Therefore, control at the protein translation level plays a critical role in gene regulation. The process of translation can be divided into initiation, elongation, termination, and ribosome recycling [3]. Most of the regulation of protein translation is exerted at the initiation phase, allowing for a rapid and reversible control of gene expression [1, 3].

Initiation of Protein Translation

Protein synthesis initiation requires assembly of ribosomal subunits that are competent for translation elongation in which the anti-codon loop of initiator tRNA (Met-tRNA$^{\text{Met}}{}_{\text{i}}$) base pairs with the initiation codon of an mRNA [1, 3]. This process uses nine different eukaryotic initiation factors (eIFs). A ternary complex (TC) is first formed comprising a 40S ribosomal subunit, eIF2-GTP, and Met-tRNA$^{\text{Met}}{}_{\text{i}}$. This transforms the TC into a 43S pre-initiation complex (PIC) by complexing with certain other eIFs and binding to the capped 5′ proximal region of mRNA. This step involves additional eIFs that are needed to unwind the secondary structure of the mRNA [1]. The 43S complex scans the 5′ untranslated region (5′ UTR) of mRNA in the 5′ to 3′ direction until it recognizes the initiation codon. Once the initiation codon is recognized and the 48S complex is formed, eIF5 and eIF5B promote hydrolysis of the eIF2-bound GTP, the displacement of eIFs, and joining of a 60S ribosomal subunit.

Unfolded Protein Response

The unfolded protein response (UPR) is a collection of adaptive feedback mechanisms induced following the accumulation of excess unfolded proteins in the lumen of endoplasmic reticulum (EnR) (known as EnR stress) in cells with a high load of protein synthesis [8]. UPR is comprised of three distinct sensors that are activated following EnR stress, namely, **i**nositol **re**quiring protein 1 alpha (IRE1-α), **a**ctivating **t**ranscription **f**actor 6 (ATF6), and **p**rotein kinase RNA-like **e**ndoplasmic **r**eticulum **k**inase (PERK). These sensors activate diverse pathways collectively known as the UPR [8, 9] (Fig. 5.1). Signaling downstream from the UPR sensors coordinate complex cross-talk to restore proteostasis; this signaling is largely cyto-protective [9]. Many downstream signals are transcription factors (such as ATF6, ATF4, CHOP, CReP, XBP1s) that translocate to the nucleus and engage in the transcriptional regulation of a variety of genes that control cell fate [10]. Many of these genes

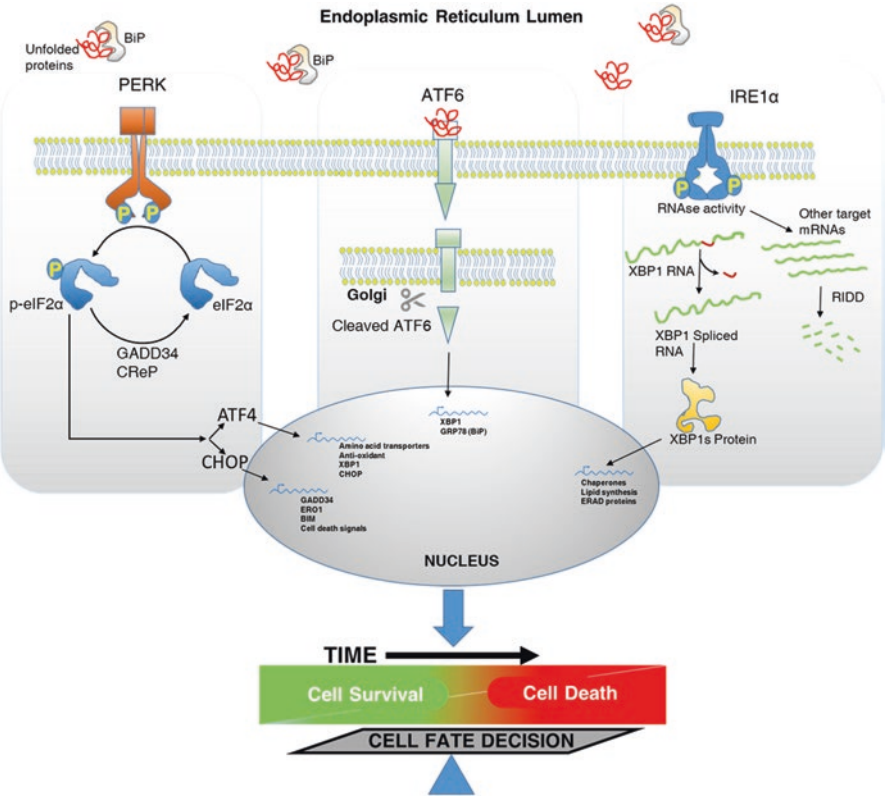

Fig. 5.1 Cartoon depicting the three arms of unfolded protein response (UPR) and its signals converging at the nucleus of the cell. The length of the time of UPR determines the cell fate

help in adaptation to stress, but some may also promote cell death. Therefore, a cell fate decision in favor of adaptive survival or cell death is a consequence of the integrated temporal response initiated by the three distinct sensors of EnR stress over time. For example, chronic and unmitigated UPR leads to apoptosis [11–14], mediated through the mitochondrial/intrinsic pathway [15].

All three UPR sensors (IRE1α, ATF6, PERK) are transmembrane proteins spanning through the EnR membrane [8]. The luminal domains of these proteins can sense an imbalance in protein folding efficiency inside the EnR and consequently trigger downstream signaling. Specifically, under basal, unstressed conditions the luminal domains of these sensor proteins bind with a chaperone, glucose-regulated protein 78 (GRP78) (also known as binding immunoglobulin protein (BiP) or heat shock protein A5 (HSPA5)) that prevents sensor activation [8, 10].

Activation of the PERK pathway hyper-phosphorylates eukaryotic translational initiation factor 2 alpha (p-eIF2α), which then inhibits global protein translation to restore proteostasis [10]. However, sustained activation of eIF2α can also lead to cell death [8, 16]. The level of eIF2α phosphorylation is tightly controlled by activity of the protein phosphatase 1 (PP1) complex by two specific regulatory subunits, (1) growth arrest and DNA damage inducible-34 (GADD34) and (2) constitutive repressor of eIF2α phosphorylation (CReP), that are responsible for dephosphorylation of eIF2α [16–18] (Fig. 5.2). The other two additional UPR sensors (ATF6 and IRE1α) also contribute to cell fate decisions by complex essential and redundant cross-talk with the PERK arm and modulate the downstream components that may have protective or apoptotic effects [19].

EIF2 Alpha Phosphorylation and Translational Regulation

Formation of the TC during the initiation of protein translation depends upon the availability of GTP-bound eIF2 and its recycling to maintain protein synthesis. Regeneration of GTP-eIF2 is ensured by another initiation factor, eIF2B, which functions as a guanine nucleotide exchange factor. The alpha subunit of eIF2 (eIF2α) can be phosphorylated on serine 51 residue by activated PERK and functions as a major regulatory checkpoint. Phospho-eIF2α can bind strongly to eIF2B and restrict its availability. While phosphorylated-eIF2α can form the TC, its higher levels block regeneration of the active GTP-bound eIF2α by sequestering eIF2B and abrogating its activity. Consequently, the low levels of GTP-eIF2α lead to reduced translation initiation and a suppression of global protein synthesis. Notably, three additional mammalian protein kinases, besides PERK (EIF2AK3) can phosphorylate eIF2α (Fig. 5.2). These three kinases are heme-regulated inhibitor kinase (EIF2AK1), which is significant only in erythroid cells [20]; PKR (EIF2AK2), which can be activated by viral response [21, 22]; and GCN2 (EIF2AK4) that is activated by amino acid starvation [23].

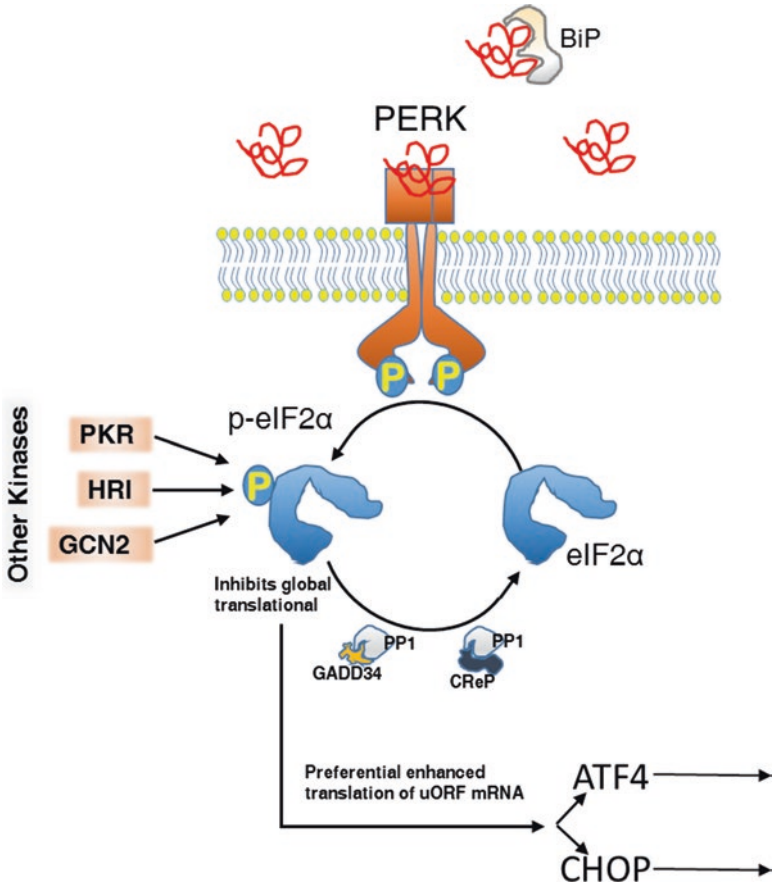

Fig. 5.2 PERK-eIF2α-ATF4/CHOP axis

Regulation of eIF2α Phosphorylation

Phosphorylation of eIF2α is a reversible process and its precise balance in critical for cell survival. To counteract the phosphorylation of eIF2α, two distinct proteins (GADD34 and CReP) function as regulatory subunits forming two distinct holocomplex with protein phosphatase 1 (PP1) to dephosphorylate eIF2α [13]. GADD34 (also known as PPP1R15A) is an inducible factor downstream of PERK activation and functions as a feedback loop [8, 13, 18, 24]. Conversely, CReP is the constitutively expressed regulatory protein of PP1 complex that dephosphorylates eIF2α and is responsible for maintaining the balance of phosphorylated and non-phosphorylated eIF2α in unstressed cells [8, 19, 25]. Activated IRE1α also cleaves other EnR mRNAs (besides XBP1) [26–29], ribosomal RNA [30], and microRNAs

[31, 32] that share specific nucleotide sequences as in XBP1 [28] in a process known as regulated IRE1α-dependent decay (RIDD). Notably, CReP mRNA is cleaved by activated IRE1α, establishing unique cross-talk between different UPR sensors that can increase phospho-eIF2α levels and reduce protein load [33].

Preferred Protein Translation of Selective Messenger RNA

The primary goal of UPR is to maintain ER proteostasis. Proteostasis is chiefly attained by global suppression of protein translation to reduce newly synthesized protein-load into EnR [34]. However, a distinct subset of messenger RNAs coding for certain proteins and transcription factors evade the global suppression of translation triggered by eIF2α phosphorylation. Paradoxically, these mRNAs are translated at a higher rate in stressed cells [35]. Most of these factors are required to respond to the consequences of EnR stress and include ATF4 [36, 37], GADD34 (PPP1R15A) [38], ATF5 [39–41], and CHOP (DDIT3) [42, 43]. The mechanism of enhanced protein synthesis of these factors is attributed to the small inhibitory upstream open reading frames (uORF) located within the 5′-leader (untranslated region) of their mRNA [44, 45]. Genome-wide ribosomal profiling has revealed that over 40% of mammalian mRNA contain uORFs that may serve as a major regulator of translation and protein levels [44, 46, 47].

Multiple mechanisms exist by which uORF-containing mRNAs can be translated preferentially in stressed cells with high levels of phospho-eIF2α [35]. The capacity to reinitiate translation from a downstream start codon, known as ribosome reinitiation, depends upon the ability of the scanning ribosome to acquire or retain the essential initiation factors following translation of uORF. The distance between the initiation codon of uORF and the initiation codon of the coding protein plays a critical role, as it allows more time to re-acquire a new eIF2-GTP-Met-tRNAimet [48]. For example, the transcription factor ATF4 mRNA contains uORFs that restrict ribosome access to the coding sequence (CDS) in unstressed cells but allows increased access and translation of the ATF4 protein in stressed cells with higher levels of phospho-eIF2α [36, 37]. This occurs because reduced eIF2α-GTP levels in stressed cells delay reinitiation of ribosomes that enables skipping of the inhibitory uORFs and increases ribosome access to the start codon of the ATF4 coding sequence [37].

Another mechanism by which preferential translation is regulated in stressed cells with high levels of phospho-eIf2α is reported for the GADD34 protein [49], which functions as a feedback control for eIF2α dephosphorylation [24, 50, 51]. The GADD34 mRNA contains two uORFs. uORF1 is constitutive but is bypassed due to a poor kozak sequence. uORF2 is the main inhibitory sequence as deletion of this uORF increases the expression of GADD34 [49]. Under stress conditions, the

ribosome bypasses uORF2 due to "poor start codon context," allowing higher translation from the CDS start codon [49]. This mechanism is also evident during the preferential translation of CHOP in chronically stressed cells [43, 52]. Expression of CHOP protein in cells with unmitigated stress and continued elevated levels of phospho-eIF2α can induce apoptosis [53–55]. Notably, GADD34, is regulated at the translational level and its rate of transcription is increased by ATF4 [56] and CHOP [57]. Both ATF4 and CHOP are preferentially translated in response to EnR stress and hyper-phosphorylation of eIF2α [37, 52] indicating a coordinated mechanism to remediate stress.

A recent study shows a new mechanism that relies on eIF2α independent and non-AUG starting codon in the uORF to regulate translation in stressed cells [58]. This study discovered that an alternative initiation factor, eIF2A, and non-AUG ORF is required for translation of GRP78 (HSPA5; BiP) during stress response [58]. In addition, the internal ribosome entry sequences (IRES) may also play a role in expression of GRP78 protein [59].

Role of PERK in Breast Cancer

Multiple studies have implicated a central role for the UPR in several cancers including breast cancers [60]. In breast cancers, integration of UPR, EnR stress, and autophagy drives the cell fate in endocrine therapy resistance [10]. For example, depletion of estrogen receptor [61] and GRP78 [62], a key component of the UPR pathway, restored endocrine sensitivity in the endocrine therapy resistant breast cancer cells. Inhibition of autophagy potentiates antiestrogen therapy in the resistant breast cancers [63]. In addition, XBP1, another key component of UPR, not only contributes to estrogen-mediated cell proliferation [64] but also plays a vital role in conferring endocrine resistance upon breast cancer cells [65–68].

In particular, the PERK pathway plays a critical role in oncogenic development, survival, progression, and invasion of cancers [69–73]. In breast cancers, PERK signaling is associated with invasion and metastasis [74], and selectively sensitizes cancer cells that have undergone an epithelial-to-mesenchymal transition to an EnR stress (EMT) [75].

High doses of estrogen were used as a therapy for estrogen receptor positive breast cancers before the discovery antiestrogens [76, 77]. Laboratory studies have confirmed that estrogen can induce apoptotic cell death in select LTED (long-term estrogen-deprived) breast cancer cells, both in vitro and in vivo [78, 79]. UPR, specifically, the PERK-eIF2α axis, is involved in estrogen induced apoptosis [80–82]. Studies have confirmed that prolonged and unmitigated phosphorylation of eIF2α can induce apoptosis in endocrine therapy resistant breast cancer cells using the same estrogen-mediated mechanism (Sengupta et al. in press, 2019).

Conclusions and Future Direction

Protein translation control has emerged as a critical mechanism to maintain the integrity of cells and allows them to adapt to numerous stress. Increasingly, this control is being recognized as a critical feature in development and various diseases including several cancers. Precise understanding of mechanisms governing the preferential translation of numerous proteins after activation of the PERK-eIF2α pathway will be immensely helpful in determining the factors responsible for cell fate decisions. In addition, the cross-talk between the different components of UPR pathway and its influence on the PERK pathway may predict a prosurvival or prodeath outcome. Clearly, further studies are needed to develop a unified model integrating all components of UPR signaling and its role in cell fate determination.

References

1. Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. Nat Rev Mol Cell Biol. 2010;11(2):113–27.
2. Schuller AP, Green R. Roadblocks and resolutions in eukaryotic translation. Nat Rev Mol Cell Biol. 2018;19(8):526–41.
3. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell. 2009;136(4):731–45.
4. Anelli T, Sitia R. Protein quality control in the early secretory pathway. EMBO J. 2008;27(2):315–27.
5. Reid DW, Nicchitta CV. Diversity and selectivity in mRNA translation on the endoplasmic reticulum. Nat Rev Mol Cell Biol. 2015;16(4):221–31.
6. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. Science. 2008;319(5865):916–9.
7. Liu TY, Huang HH, Wheeler D, Xu Y, Wells JA, Song YS, Wiita AP. Time-resolved proteomics extends ribosome profiling-based measurements of protein synthesis dynamics. Cell Syst. 2017;4(6):636–644.e9.
8. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334(6059):1081–6.
9. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007;8(7):519–29.
10. Clarke R, Cook KL, Hu R, Facey CO, Tavassoly I, Schwartz JL, Baumann WT, Tyson JJ, Xuan J, Wang Y, Warri A, et al. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. Cancer Res. 2012;72(6):1321–31.
11. Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, Gotoh T, et al. ER stress triggers apoptosis by activating BH3-only protein Bim. Cell. 2007;129(7):1337–49.
12. Sano R, Reed JC. ER stress-induced cell death mechanisms. Biochim Biophys Acta. 2013;1833(12):3460–70.
13. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Biol. 2011;13(3):184–90.
14. Woehlbier U, Hetz C. Modulating stress responses by the UPRosome: a matter of life and death. Trends Biochem Sci. 2011;36(6):329–37.
15. Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov. 2008;7(12):1013–30.

16. Moreno JA, Radford H, Peretti D, Steinert JR, Verity N, Martin MG, Halliday M, Morgan J, Dinsdale D, Ortori CA, Barrett DA, et al. Sustained translational repression by eIF2alpha-P mediates prion neurodegeneration. Nature. 2012;485(7399):507–11.
17. Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D, Yuan J. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science. 2005;307(5711):935–9.
18. Kojima E, Takeuchi A, Haneda M, Yagi A, Hasegawa T, Yamaki K, Takeda K, Akira S, Shimokata K, Isobe K. The function of GADD34 is a recovery from a shutoff of protein synthesis induced by ER stress: elucidation by GADD34-deficient mice. FASEB J. 2003;17(11):1573–5.
19. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016;529(7586):326–35.
20. Lu L, Han AP, Chen JJ. Translation initiation control by heme-regulated eukaryotic initiation factor 2alpha kinase in erythroid cells under cytoplasmic stresses. Mol Cell Biol. 2001;21(23):7971–80.
21. Galabru J, Katze MG, Robert N, Hovanessian AG. The binding of double-stranded RNA and adenovirus VAI RNA to the interferon-induced protein kinase. Eur J Biochem. 1989;178(3):581–9. .
22. Meurs E, Chong K, Galabru J, Thomas NS, Kerr IM, Williams BR, Hovanessian AG. Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. Cell. 1990;62(2):379–90.
23. Berlanga JJ, Santoyo J, De Haro C. Characterization of a mammalian homolog of the GCN2 eukaryotic initiation factor 2alpha kinase. Eur J Biochem. 1999;265(2):754–62.
24. Connor JH, Weiser DC, Li S, Hallenbeck JM, Shenolikar S. Growth arrest and DNA damage-inducible protein GADD34 assembles a novel signaling complex containing protein phosphatase 1 and inhibitor 1. Mol Cell Biol. 2001;21(20):6841–50.
25. Jousse C, Oyadomari S, Novoa I, Lu P, Zhang Y, Harding HP, Ron D. Inhibition of a constitutive translation initiation factor 2alpha phosphatase, CReP, promotes survival of stressed cells. J Cell Biol. 2003;163(4):767–75.
26. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol. 2009;186(3):323–31.
27. Hollien J, Weissman JS. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. Science. 2006;313(5783):104–7.
28. Oikawa D, Tokuda M, Hosoda A, Iwawaki T. Identification of a consensus element recognized and cleaved by IRE1 alpha. Nucleic Acids Res. 2010;38(18):6265–73.
29. Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, Backes BJ, Oakes SA, Papa FR. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell. 2009;138(3):562–75.
30. Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, Kimata Y, Tsuru A, Kohno K. Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. Nat Cell Biol. 2001;3(2):158–64.
31. Lerner AG, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, Igbaria A, Shen S, Nguyen V, Backes BJ, Heiman M, Heintz N, et al. IRE1alpha induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab. 2012;16(2):250–64.
32. Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, Truitt M, McManus MT, Ruggero D, Goga A, Papa FR, et al. IRE1alpha cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. Science. 2012;338(6108):818–22.
33. So JS, Cho S, Min SH, Kimball SR, Lee AH. IRE1alpha-dependent decay of CReP/Ppp1r15b mRNA increases eukaryotic initiation factor 2alpha phosphorylation and suppresses protein synthesis. Mol Cell Biol. 2015;35(16):2761–70.
34. Hetz C, Chevet E, Oakes SA. Proteostasis control by the unfolded protein response. Nat Cell Biol. 2015;17(7):829–38.

35. Young SK, Wek RC. Upstream open Reading frames differentially regulate gene-specific translation in the integrated stress response. J Biol Chem. 2016;291(33):16927–35.
36. Lu PD, Harding HP, Ron D. Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. J Cell Biol. 2004;167(1):27–33.
37. Vattem KM, Wek RC. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc Natl Acad Sci U S A. 2004;101(31):11269–74.
38. Lee YY, Cevallos RC, Jan E. An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation. J Biol Chem. 2009;284(11):6661–73.
39. Hatano M, Umemura M, Kimura N, Yamazaki T, Takeda H, Nakano H, Takahashi S, Takahashi Y. The 5′-untranslated region regulates ATF5 mRNA stability via nonsense-mediated mRNA decay in response to environmental stress. FEBS J. 2013;280(18):4693–707.
40. Watatani Y, Ichikawa K, Nakanishi N, Fujimoto M, Takeda H, Kimura N, Hirose H, Takahashi S, Takahashi Y. Stress-induced translation of ATF5 mRNA is regulated by the 5′-untranslated region. J Biol Chem. 2008;283(5):2543–53.
41. Zhou D, Palam LR, Jiang L, Narasimhan J, Staschke KA, Wek RC. Phosphorylation of eIF2 directs ATF5 translational control in response to diverse stress conditions. J Biol Chem. 2008;283(11):7064–73.
42. Chen YJ, Tan BC, Cheng YY, Chen JS, Lee SC. Differential regulation of CHOP translation by phosphorylated eIF4E under stress conditions. Nucleic Acids Res. 2010;38(3):764–77.
43. Jousse C, Bruhat A, Carraro V, Urano F, Ferrara M, Ron D, Fafournoux P. Inhibition of CHOP translation by a peptide encoded by an open reading frame localized in the chop 5'UTR. Nucleic Acids Res. 2001;29(21):4341–51.
44. Baird TD, Palam LR, Fusakio ME, Willy JA, Davis CM, McClintick JN, Anthony TG, Wek RC. Selective mRNA translation during eIF2 phosphorylation induces expression of IBTKalpha. Mol Biol Cell. 2014;25(10):1686–97.
45. Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science. 2009;324(5924):218–23.
46. Calvo SE, Pagliarini DJ, Mootha VK. Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. Proc Natl Acad Sci U S A. 2009;106(18):7507–12.
47. Fritsch C, Herrmann A, Nothnagel M, Szafranski K, Huse K, Schumann F, Schreiber S, Platzer M, Krawczak M, Hampe J, Brosch M. Genome-wide search for novel human uORFs and N-terminal protein extensions using ribosomal footprinting. Genome Res. 2012;22(11):2208–18.
48. Kozak M. Effects of intercistronic length on the efficiency of reinitiation by eucaryotic ribosomes. Mol Cell Biol. 1987;7(10):3438–45.
49. Young SK, Willy JA, Wu C, Sachs MS, Wek RC. Ribosome reinitiation directs gene-specific translation and regulates the integrated stress response. J Biol Chem. 2015;290(47):28257–71.
50. Harding HP, Zhang Y, Scheuner D, Chen JJ, Kaufman RJ, Ron D. Ppp1r15 gene knockout reveals an essential role for translation initiation factor 2 alpha (eIF2alpha) dephosphorylation in mammalian development. Proc Natl Acad Sci U S A. 2009;106(6):1832–7.
51. Novoa I, Zeng H, Harding HP, Ron D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. J Cell Biol. 2001;153(5):1011–22.
52. Palam LR, Baird TD, Wek RC. Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. J Biol Chem. 2011;286(13):10939–49.
53. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev. 2004;18(24):3066–77.
54. Teske BF, Fusakio ME, Zhou D, Shan J, McClintick JN, Kilberg MS, Wek RC. CHOP induces activating transcription factor 5 (ATF5) to trigger apoptosis in response to perturbations in protein homeostasis. Mol Biol Cell. 2013;24(15):2477–90.

55. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev. 1998;12(7):982–95.
56. Ma Y, Hendershot LM. Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. J Biol Chem. 2003;278(37):34864–73.
57. Ma Y, Brewer JW, Diehl JA, Hendershot LM. Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. J Mol Biol. 2002;318(5):1351–65.
58. Starck SR, Tsai JC, Chen K, Shodiya M, Wang L, Yahiro K, Martins-Green M, Shastri N, Walter P. Translation from the 5′ untranslated region shapes the integrated stress response. Science. 2016;351(6272):aad3867.
59. Cho S, Park SM, Kim TD, Kim JH, Kim KT, Jang SK. BiP internal ribosomal entry site activity is controlled by heat-induced interaction of NSAP1. Mol Cell Biol. 2007;27(1):368–83.
60. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer. 2014;14(9):581–97.
61. Cook KL, Clarke PA, Parmar J, Hu R, Schwartz-Roberts JL, Abu-Asab M, Warri A, Baumann WT, Clarke R. Knockdown of estrogen receptor-alpha induces autophagy and inhibits antiestrogen-mediated unfolded protein response activation, promoting ROS-induced breast cancer cell death. FASEB J. 2014;28(9):3891–905.
62. Cook KL, Shajahan AN, Warri A, Jin L, Hilakivi-Clarke LA, Clarke R. Glucose-regulated protein 78 controls cross-talk between apoptosis and autophagy to determine antiestrogen responsiveness. Cancer Res. 2012;72(13):3337–49.
63. Cook KL, Warri A, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, Clarke R. Hydroxychloroquine inhibits autophagy to potentiate antiestrogen responsiveness in ER+ breast cancer. Clin Cancer Res. 2014;20(12):3222–32.
64. Sengupta S, Sharma CG, Jordan VC. Estrogen regulation of X-box binding protein-1 and its role in estrogen induced growth of breast and endometrial cancer cells. Horm Mol Biol Clin Investig. 2010;2(2):235–43.
65. Gomez BP, Riggins RB, Shajahan AN, Klimach U, Wang A, Crawford AC, Zhu Y, Zwart A, Wang M, Clarke R. Human X-box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. FASEB J. 2007;21(14):4013–27.
66. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang HB, Clarke R. NF-kappaB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol. 2015;35(2):379–90.
67. Shajahan AN, Riggins RB, Clarke R. The role of X-box binding protein-1 in tumorigenicity. Drug News Perspect. 2009;22(5):241–6.
68. Zhu Y, Singh B, Hewitt S, Liu A, Gomez B, Wang A, Clarke R. Expression patterns among interferon regulatory factor-1, human X-box binding protein-1, nuclear factor kappa B, nucleophosmin, estrogen receptor-alpha and progesterone receptor proteins in breast cancer tissue microarrays. Int J Oncol. 2006;28(1):67–76.
69. Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, Koumenis C, Harding HP, Ron D, Holcik M, Bell JC. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol Cell Biol. 2006;26(24):9517–32.
70. Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, Zhang F, Ye J, Koumenis C, Cavener D, Diehl JA. PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. Oncogene. 2010;29(27):3881–95.
71. Bu Y, Diehl JA. PERK integrates oncogenic signaling and cell survival during cancer development. J Cell Physiol. 2016;231(10):2088–96.
72. Dey S, Sayers CM, Verginadis II, Lehman SL, Cheng Y, Cerniglia GJ, Tuttle SW, Feldman MD, Zhang PJ, Fuchs SY, Diehl JA, et al. ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis. J Clin Invest. 2015;125(7):2592–608.
73. Nagelkerke A, Bussink J, Mujcic H, Wouters BG, Lehmann S, Sweep FC, Span PN. Hypoxia stimulates migration of breast cancer cells via the PERK/ATF4/LAMP3-arm of the unfolded protein response. Breast Cancer Res. 2013;15(1):R2.

74. Feng YX, Jin DX, Sokol ES, Reinhardt F, Miller DH, Gupta PB. Cancer-specific PERK signaling drives invasion and metastasis through CREB3L1. Nat Commun. 2017;8(1):1079.
75. Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q, Gupta PB. Epithelial-to-mesenchymal transition activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress. Cancer Discov. 2014;4(6):702–15.
76. Haddow A, Watkinson JM, Paterson E, Koller PC. Influence of synthetic oestrogens on advanced malignant disease. Br Med J. 1944;2(4368):393–8.
77. Peethambaram PP, Ingle JN, Suman VJ, Hartmann LC, Loprinzi CL. Randomized trial of diethylstilbestrol vs. tamoxifen in postmenopausal women with metastatic breast cancer. An updated analysis. Breast Cancer Res Treat. 1999;54(2):117–22.
78. Lewis JS, Meeke K, Osipo C, Ross EA, Kidawi N, Li T, Bell E, Chandel NS, Jordan VC. Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation. J Natl Cancer Inst. 2005;97(23):1746–59.
79. Song RX, Mor G, Naftolin F, McPherson RA, Song J, Zhang Z, Yue W, Wang J, Santen RJ. Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17beta-estradiol. J Natl Cancer Inst. 2001;93(22):1714–23.
80. Fan P, Griffith OL, Agboke FA, Anur P, Zou X, McDaniel RE, Creswell K, Kim SH, Katzenellenbogen JA, Gray JW, Jordan VC. c-Src modulates estrogen-induced stress and apoptosis in estrogen-deprived breast cancer cells. Cancer Res. 2013;73(14):4510–20.
81. Fan P, Tyagi AK, Agboke FA, Mathur R, Pokharel N, Jordan VC. Modulation of nuclear factor-kappa B activation by the endoplasmic reticulum stress sensor PERK to mediate estrogen-induced apoptosis in breast cancer cells. Cell Death Discov. 2018;4:15.
82. Ariazi EA, Cunliffe HE, Lewis-Wambi JS, Slifker MJ, Willis AL, Ramos P, Tapia C, Kim HR, Yerrum S, Sharma CG, Nicolas E, et al. Estrogen induces apoptosis in estrogen deprivation-resistant breast cancer through stress responses as identified by global gene expression across time. Proc Natl Acad Sci U S A. 2011;108(47):18879–86.

Chapter 6
Roles of Spliced and Unspliced XBP1 in Breast Cancer

Rong Hu and Robert Clarke

Abstract XBP1 is a critical determinant of several outcomes following activation of the unfolded protein response (UPR). This UPR gene is initially transcribed as an unspliced mRNA but can subsequently be spliced by the endoribonuclease activity of IRE1α induced by activation of GRP78 in response to endoplasmic reticulum stress. Both the unspliced (XBP1-U) and spliced (XBP1-S) mRNAs are translated into proteins. XBP1-U, which cannot function as a transcription factor, can act as a dominant negative regulator of XBP1-S. In contrast, the frameshift produced by the removal of 26 bp intron from an already matured XBP1 mRNA, produces a transcription factor (XBP1-S). This chapter discusses the regulation and unconventional splicing of XBP1 and the roles of both the unspliced and spliced proteins in breast cancer, with a focus on those breast cancers expressing the estrogen receptor.

Keywords Acetylation · Autophagy · Bcl2 · Beclin1 · EMT · Endoplasmic reticulum stress · GRP78 · IRE1α · p300 · PERK · Phosphorylation · SIRT1 · Splicing · Sumoylation

In mammalian cells, three ER transmembrane proteins (IRE1α, PERK, and ATF6) play critical roles in mediating the unfolded protein response [1, 2]. Under normal conditions, the ER lumen domain of these three proteins is occupied by molecular chaperones such as GRP78 and thus remain inactive [3]. Once cells undergo ER stress, GRP78 is released from these proteins to facilitate proper protein folding [4]. These key proteins become active and send downstream signaling for cells to combat ER stress [3]. Thus, these three proteins are also called ER stress sensors.

R. Hu (✉)
Department of Oncology, Georgetown Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA
e-mail: rh335@georgetown.edu

R. Clarke
Department of Oncology, Georgetown University Medical Center, Washington, DC, USA
e-mail: clarker@georgetown.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_6

Among the three sensors of the UPR, IRE1α is the most conserved transducer [5]. Upon ER stress, IRE1α activation results in the production of one of its key downstream targets, XBP1-S [6]. XBP1-S is a basic-region leucine zipper (bZIP) protein. It was named X-box binding protein because it was first discovered to be bound to the cis-acting X-box present in the promoter regions of human major histocompatibility complex class II genes [7]. XBP1-S is a central executor of the UPR and its level of express is critical to both UPR activation and termination [8]. As a transcription factor, XBP1-S translocates to the nucleus and induces transcription of its target genes [9]. Downstream target functions of the IRE1α-XBP1 pathway include ER membrane biosynthesis, protein transportation, secretory machinery of exocrine glands, ER chaperones, lipid synthesis, ERAD (II), ER translation and inflammation [9]. Upregulation of these activities enhances the ER's capacity to better fold new proteins and translocate irreparable proteins from ER to cytosol for proteasomal degradation [1].

XBP1 Unconventional Splicing

IRE1α activates the production of XBP1-S through an unconventional RNA splicing mechanism, which is fundamentally different from conventional RNA splicing in several aspects. Conventional splicing is the editing process that removes introns from newly transcribed RNA (pre-mRNA) and re-joins exons to form mature mRNA [10]. Almost all mRNA, including XBP1, undergo conventional RNA splicing before they can be translated into protein [10]. Unconventional splicing of XBP1 removes a 26 bp intron from an already mature XBP1 mRNA [11].

Conventional RNA splicing occurs inside the nucleus, often immediately after an RNA is transcribed [10]. XBP1, in contrast, unconventional splicing occurs mainly in the cytoplasm, but can be also observed in the nucleus [6]. ER stress induced splicing of XBP1 occurs in the cytoplasm, whereas the basal XBP1 splicing of its immature transcript occurs in the nucleus [12]. Both unspliced (but mature XBP1 mRNA (XBP1-U) and unconventionally spliced XBP1 mRNA (XBP1-S) can be translated into their respective proteins. Nascent XBP1-U polypeptides recruit their own mRNA to the ER membrane through a hydrophobic region within XBP1-U, pulling the XBP1 mRNA close to IRE1α as a substrate for unconventional splicing [12].

Third, conventional RNA splicing is carried out by spliceosomes, a complex of small nuclear ribonucleoproteins (snRNPs) inside the nucleus [10]. In contrast, XBP1 unconventional splicing is independent of snRNPs and carried out by the endonuclear function of the IRE1α protein [11, 13]. The N-terminus IRE1α ER luminal domain is located inside the ER lumen, whereas the Ser/Thr Kinase domain and its C-terminal RNase domain are both located on the cytosolic side of the ER membrane. Accumulation of unfolded proteins inside the ER lumen induces IRE1α dimerization and oligomerization of [4, 14]. Trans-autophosphorylation of IRE1α in the Ser/Thr kinase domain, a consequence of dimerization, leads to activation of

the distal RNase domain, that recognizes and removes a stem-loop structure on the XBP1 mRNA [15].

In addition to processing the *XBP1* mRNA, IRE1α cleaves other mRNAs or microRNAs (miRs) localized to the ER membrane and subsequently leads to their degradation, a process known as regulated IRE1α-dependent decay (RIDD), which also plays a critical role in UPR [16, 17]. Recent research suggests that IRE1α-mediated cleavage of XBP1 and RIDD of mRNAs or miRs are separable activities that are associated with distinct IRE1α conformational changes and affinities to the substrates [18].

XBP1-S and XBP1-U Post-Translational Regulation

Prior to its unconventional splicing, XBP1 mRNA produces a 29 kDa XBP1-U (XBP1-unspliced) protein. Under basal conditions, XBP1-U is the main form inside a cell. XBP1-U protein has a rapid turnover [15]. Indeed, the rate of XBP1-U degradation can approach its rate of synthesis. Thus, while XBP1-U is constitutively synthesized, the steady state expression level of XBP1-U under basal conditions is low. The C-terminal of XBP1-U is critical for the fast turnover and involves both ubiquitin-dependent and ubiquitin-independent mechanisms [15]. In addition, XBP1-U interacts with purified 20S proteasomes through its unstructured C-terminus, which leads to XBP1-U degradation in a manner that autonomously opens the proteasome gate [19].

Under ER stress, IRE1α removes a 26 bp intron from the XBP1 mRNA that produces a frameshift and generation of a 56 kDa XBP1-S (XBP1-spliced) protein with a distinct sequence [15]. The two forms of XBP1 share the same N-terminus; both have the basic-region leucine zipper (bZIP) motif that is responsible for DNA binding and dimerization. However, the C-termini are distinct and have different functional domains. XBP1-S contains a transcriptional activation domain and thus can act as a potent transcription factor. In contrast, the C-terminus of XBP1-U retains a nuclear exclusion signal and thus its role is less clear.

The XBP1-S protein is much more stable than the XBP1-U protein. Degradation of XBP1-S is proteasome dependent [20]. The degradation of XBP1-S is controlled by UBC9, which specifically binds to the bZIP motif of XBP1-S and increases its stability [20]. Since XBP1-U shares the bZIP motif required for UBC9 binding, UBC9 also binds to XBP1-U, but less efficiently [20]. The C-terminal portion of XBP1-U may inhibit its interaction with UBC9. Interaction with UBC9 protects XBP1-S from degradation by the proteasome [20]. Interestingly, while UBC9 is commonly known as a SUMO-conjugase, its conjugase activity is dispensable for UBC9-dependent XBP1-S degradation [20]. It is unclear whether the degradation of XBP1-S is regulated through its post-translational modulation.

XBP1-S is known to be modulated post-translation via sumoylation and acetylation [21, 22]. Sumoylation is mediated by PIAS2 at two lysine residues, K297 and K276, of the C-terminal transactivation domain of XBP1-S [21]. The sumoylation

of K297 is required for K276 sumoylation, as K297R mutation prevents XBP1-S sumoylation on both sites. The sumoylation of XBP1-S represses its transcriptional activity but does not alter its nuclear localization [21]. XBP1-S sumoylation is observed at both basal condition and under ER stress, and the signaling that regulates XBP1-S sumoylation is not clear [21]. It has been shown that XBP1-S can be acetylated by p300 and deacetylated by SIRT1, which enhances and inhibits XBP1-S transcriptional activity, respectively [22]. The interaction between p300 seems to be specific to XBP1-S, as no interaction has been detected with XBP1-U [22]. However, the lysine residues involved in XBP1-S acetylation have yet to be mapped out to determine whether acetylation and sumoylation regulates XBP1-S transcriptional activity in a competitive manner. Interestingly, the presence of p300 also stabilizes XBP1-S, suggesting the acetylation of XBP1-S prevents its protein degradation [22]. The underlying mechanism of this protection is yet to be determined.

Protein Function of XBP1-S and XBP1-U

XBP1-S Role in UPR

Upon ER stress, XBP1-S upregulates a variety of genes involved in ER membrane biosynthesis, chaperones, and ERAD [9]. XBP1-S recognizes and binds to the ER stress response element (ERSE) and the UPR element (UPRE) in the promoter region of target genes such as GRP78, and ERdj3. These XBP1-S target genes then reduce ER stress. For example, ER capacity is expanded, and misfolded protein degradation chaperone expression is increased and generally enables increased protein throughput [8]. Cells lacking XBP1-S showed an impaired ability to produce UPR target genes and to activate an ER stress response [8].

XBP1-S can also send feedback signals to its upstream regulator IRE1α [23]. A positive feedback loop between XBP1-S and IRE1α has been reported. The expression and phosphorylation of IRE1α are both regulated by XBP1-S [23]. In addition, IRE1α-XBP1 can crosstalk with other UPR signaling pathways. For example, XBP1 activates the PERK-ATF4 pathway through inducing NCOA3 expression in breast cancer cells [24].

In addition to the UPR response, XBP1-S is involved in autophagy, a cellular mechanism that is tightly linked to UPR response [1]. Autophagy is a conserved pathway that involves lysosomal degradation of proteins and organelles to promote cell viability under stress conditions [1]. Autophagy could also be pro-death if the stress condition cannot be overcome by the cells. XBP1-S interacts with the autophagy regulator, Beclin 1, and thus promotes macrophage survival and autophagy [25]. Furthermore, XBP1 splicing upregulates the expression of Beclin1 in endothelial cells and results in an autophagic response [26]. XBP1-S forms a homo- or heterodimer on the promoter region of Beclin 1 and activates Beclin 1 transcription [26]. In addition, XBP1-S has also been shown to induce autophagy indirectly

through regulating the expression of Bcl-2 [27]. The anti-apoptotic protein Bcl-2 is known to form a complex with Beclin 1 and inhibit the nucleation of autophagosome [28]. Another mechanism by which IRE1α/XBP1 controls the induction of autophagy during the UPR is by regulating the expression level of the ER membrane transporter SLC33A1/AT-1 and Atg9A acetylation [29]. Through the control of FoxO1, also a critical regulator of autophagy [30]. It has been shown that XBP1-S binds to FoxO1 via its N-terminal domain, directing FoxO1 for proteasomal degradation [30].

Roles of XBP1-S Outside of UPR

In addition to mediating UPR and downstream autophagy, recent studies revealed additional roles of XBP1-S in cellular functions that include EMT and angiogenesis [31–34]. Epithelial-mesenchymal transition (EMT) is a key process contributing to the aggressiveness of cancer cells. XBP1-S activates EMT in response to ER stress [62]. Several EMT related transcription factors including SNAI1, SNAI2, ZEB2, and TCF3 are direct transcriptional targets of XBP1 [31]. XBP1 induces MMP-9 expression to promote proliferation and invasion in esophageal squamous cell carcinoma [33]. In addition, XBP1 can regulate the expression of β-catenin, a critical effector in Wnt-mediated pathway associated with EMT [32]. It has been shown that XBP1-S bind directly to the promoter region of β-catenin [32].

XBP1-S has also been shown to play a role in angiogenesis [34, 35]. Endothelial cell-specific knockout of XBP1 in mice retarded the retinal vasculogenesis in the first 2 postnatal weeks and impaired the angiogenesis triggered by ischemia. The role of XBP1 in angiogenesis is mainly mediated through XBP1-S, as reconstitution of XBP1 by Ad-XBP1-S gene transfer significantly improved angiogenesis in ischemic tissue in XBP1 conditional knockout mice [34].

Interaction with Other Transcription Factors

As a transcription factor, XBP1-S has been reported to form a complex with other transcription factors and thus modulate each other's transcriptional activity. The first transcription factor that has been shown to interact directly with XBP1-S is ERα [36]. XBP1-S binds to ERα via the XBP1 bZIP domain. This occurs in an estrogen-independent manner [36]. ERα also binds to XBP1-U, which also contains the bZIP domain but with a lower affinity [36, 37]. Binding of XBP1-S results in enhanced ERα-dependent transcriptional activity. It is unknown whether XBP1-S transcriptional activity is altered by ERα binding.

Subsequently, it has been shown that the transcription factor HIF1α can also interact with XBP1-S [38]. XBP1-S physically interacts with HIF1α also via its amino-terminal bZIP domain [38]. Unlike ERα binding, HIF1α does not bind to

XBP1-U, despite the bZIP domain also being present in XBP1-U [38]. Perhaps the conformation of XBP1-U prevents HIF1α binding. XBP1-S and HIF1α co-occupy and activate transcriptional targets of HIF1α, such as VEGFA, PDK1, GLUT1, and DDIT4 [38].

Another transcription factor that has been shown to interact with XBP1-S is MIST1 [39]. MIST1 is a basic helix-loop-helix transcription factor that is critical in a wide variety of secretory cell functions and responses to stress. Recently, research reveals a feedback loop between MIST1 and XBP1 [39]. ER stress induces MIST1 via XBP1. It has been shown that XBP1-S activates MIST1 gene expression. As ER stress subsides, MIST1 binds to XBP1 promoters and represses XBP1 transcription [39]. More importantly, genome wide DNA binding studies reveal that MIST1 functions as a co-regulator of XBP1 for a portion of target genes that contain adjacent MIST1 and XBP1 binding sites [39]. It has been shown that 70% of the identified XBP1 effector genes contained MIST1 peaks within their respective control regions or within the control regions of closely related proteins of the same family [39]. However, whether XBP1-S directly binds to MIST1 is unknown.

In addition to transcription factors, other binding partners for XBP1-S have been identified. XBP1-S has also been shown to be specifically bound to HDAC5 [21]. However, the effects of this interaction have yet to be determined. Even though many binding partners for XBP1-S have been identified, the mechanism by which these interactions are regulated is not known yet. XBP1-S contains many potential phosphorylation sites, it would be interesting to know whether these binding partner interactions are phosphorylation specific.

XBP1-U Role in UPR

XBP1-S is a key executor of the ER stress response, whereas XBP1-U plays an active role in fine-tuning the ER stress response, mainly through negating the effects of XBP1-S [40]. One mechanism by which XBP1-U does so is by forming a complex with XBP1-S and repressing the transcriptional activity of XBP1-S [40]. For example, the transcription of iNOS mediated by XBP1-S is repressed when XBP1-U is present [41]. Another proposed role for XBP1-U is enhancing the degradation and nuclear exclusion of XBP1-S [6, 40].

The fast turnover and nuclear export properties of XBP1-U are critical for both the accurate initiation and termination of an ER stress response [15]. XBP1-U mutants that are more stable resulted in a stronger ER stress response than wild-type XBP1-U, suggesting fast degradation of XBP1-U is required to prevent uncontrolled activation of UPR [15]. In addition, XBP1-U accumulates during the recovery phase of the ER stress, forming a complex with XBP1-S and translocating XBP1-S out of the nucleus [15]. The degradation motif contained in XBP1-U results in the rapid degradation of XBP1-S in the complex. XBP1-U functions as a negative feedback regulator of XBP1-S, shutting off XBP1-S and its downstream target genes during the recovery phase of ER stress [40]. Furthermore, XBP1-U plays an

important role in controlling the expression of IRE1α, which sends a feedback signal to UPR pathways [42].

Similar to XBP1-S, XBP1-U has a role in autophagy. Instead of promoting autophagy, XBP1-U inhibits persistent autophagy activation [43]. One mechanism by which XBP1-U regulates autophagy might be through its inhibition of XBP1-S. XBP1-U may also control autophagy through promoting the degradation of FoxO1 [43]. FoxO1 is involved in the induction of autophagy via both transcription-dependent and -independent pathways [44]. Loss of FoxO1 can also attenuate autophagy. Knockdown of XBP1-U results in the sustained expression of FoxO1 [43]. It has been shown that ERK1/2 phosphorylates XBP1-U on Ser61 and Ser176 upon glutamine starvation [43]. Phosphorylated XBP1-U promotes the interaction between XBP1-U and FoxO1, and targets FoxO1 for translocation from the nucleus to the cytoplasm and the degradation by the 20S proteasome [43]. It is possible that XBP1-U accumulated at the recovery phase of ER stress sends feedback signals to inhibit both UPR and autophagy pathways, thus blocking cell death that might otherwise be induced by their persistent activation.

Similar to XBP1-S, the cellular role of XBP1-U is not limited to UPR and autophagy. XBP1-U plays a critical role in the activation of anti-oxidative reactions induced by disturbed flow in endothelial cells [45]. XBP1-U forms a complex with HDAC3 and Akt1 in the cytosol to regulate Akt1 phosphorylation and activate downstream HO-1 expression [45]. HO-1 is a stress response gene that plays a protective role against apoptosis under stress conditions such as oxidative stress, hypoxia, heavy metal ions, cytokines, and glutathione depletion [45]. In addition to direct binding to HDAC3 and Akt1, the expression of XBP1-U is essential for HDAC3 induction, but the underlying mechanism of this regulation is unclear [45].

Elevated XBP1 Signal in Cancer

Sustained IRE1-XBP1 activation has been shown to promote tumor growth and metastasis in a variety of cancer types including breast, hepatocellular carcinoma, multiple myeloma, pancreatic, and CLL [47–51]. XBP1 has been implicated in cancer cell differentiation, susceptibility to oncovirus infection, angiogenesis, and the EMT [35, 51].

XBP1-S overexpression has been observed in primary breast cancers (by IHC) [47]. High XBP1-S/XBP1-U ratio is associated with poor survival [52]. Higher levels of XBP1-U mRNA are significantly associated with survival, consistent with the hypothesis that XBP1-U acts through countering the effects of XBP1-S [52]. XBP1-S expression is highly correlated with overall survival in the ERα+ subgroup, but not in the ERα- group [53].

Several studies have demonstrated a correlation between XBP1 and ERα expression. XBP1 co-clusters with ERα in breast tumor biopsy samples (by qPCR) ([63, 64]). High degree of correlation between ERα and XBP1 mRNA was observed in

invasive ductal carcinomas [54]. Consistently, XBP1-S protein expression is correlated with ERα positivity [55]. The correlated expression between XBP1 and ERα in breast cancer suggests a transcriptional regulatory relationship between the two. Indeed, estrogen induces XBP1 expression in breast cancer MCF7 and T47D cells [55, 56]. Recruitment of ERα, SRC-1, SRC-3, and RNA polymerase II to the promoter/enhancer region of XBP1 in these cells occurs in an estrogen-dependent manner [56]. XBP1 could also regulate the expression of ERα. ERα expression is elevated in XBP1 overexpressing cells, indicating a positive feedback loop between XBP1 and ERα [27]. However, the mechanism by which XBP1 regulates ERα expression needs to be studied further.

Direct binding between XBP1 and ERα has been reported in several studies. XBP1-S and XBP1-U both bind to ERα in vitro and in vivo in an estrogen-independent manner [27, 36, 57]. The bZIP domain in XBP1-U and -S is critical for this binding [36]. This binding seems to be specific, because neither XBP1-U nor XBP1-S binds to ERβ [57]. The binding of XBP1-S and XBP1-U both enhanced ERα-dependent transcriptional activity; XBP1-S is more potent than XBP1-U [36, 57]. The XBP1-U effects on ERα transcriptional activity can be completely blocked by ICI or Tam, whereas ICI or Tam could only partially reduce the effects of XBP1-S on ERα transcription [57]. The steroid receptor co-activator SRC-1/NCoA1 Synergies with XBP1-S and XBP1-U to regulate ERα transcriptional activity [36]. It is possible that ERα and XBP-1 form complexes at an ERE to stimulate gene expression, in a similar manner to that reported between HIF1α and XBP1 [58]. The detailed mechanism for this interaction needs to be elucidated further.

XBP1 contributes to antiestrogen resistance in ERα + breast cancer cells. XBP1 expression is increased in antiestrogen resistant breast cancer cells and co-expressed with ERα. Overexpression of XBP1-S in ERα positive cells leads to estrogen-independent growth and re-sensitizes cells to tamoxifen and faslodex independent of functional p53 [27]. XBP1-S overexpression induces the expression of Bcl-2, which is otherwise downregulated by tamoxifen and faslodex [27]. In addition, overexpression of XBP1 can activate NFκB survival signaling pathway, which is critical for antiestrogen resistance [37]. Both XBP1-S and XBP1-U activate NFκB signaling in an ERα-dependent manner. However, XBP1-S, but not XBP1-U, regulates expression level of the NFκB component p65/RelA in an ERα-independent manner [37]. Inhibition of XBP1 splicing with the IRE1α inhibitor STF-083010 re-sensitizes resistant breast cancer cells to tamoxifen in vitro [53]. Co-treatment with STF-083010 and tamoxifen can significantly delay breast cancer progression in a mammary tumor xenograft model [53].

In addition to ERα + breast cancer, XBP1 also plays a crucial role in tumorigenicity and progression of triple negative breast cancer (TNBC) [38]. Silencing of XBP1 in TNBC leads to suppression of tumor initiation, progression, metastasis, and recurrence. XBP1 plays a major role in promoting oncogenesis and cancer stem cell properties. XBP1-S is involved in maintaining a cancer stem cell like $CD44^{high}CD24^{low}$ population [38]. It has been shown that XBP1 drives TNBC progression by cooperating with HIF1α. Interestingly, while XBP1 silencing also

affects luminal breast cancer growth, it does so via a mechanism not involving HIF1α.

In addition to tumor cell intrinsic effects, XBP1 is known to have a role in the tumor-associated immune system. For example, silencing XBP1 in tumor-associated dendritic cells extends host survival by enhancing T cell antitumor immunity. Thus, targeting the ER stress response concomitantly inhibits tumor growth and enhances anti-cancer immunity, offering a unique approach to cancer immunotherapy [59]. Immunotherapeutic approach for targeting XBP1 using a cocktail of HLA-A2 unspliced and spliced heteroclitic XBP1 peptides can evoke an XBP1-specific CTL with antitumor activities against solid tumors including those breast cancers that highly express XBP1 [60].

Considering the important role of XBP1 in cancers, several inhibitors that selectively block IRE1α-XBP1 activation have been developed [61]. The inhibitors designed target either the catalytic core of the RNase domain or the ATP binding site of the kinase domain of IRE1α [61]. The efficacy of these inhibitors in specifically inhibiting IRE1α activity has been evaluated in vitro and also in cancer cells [61]. Some of the inhibitors demonstrated antitumor activity in vivo with xenograft animal model. However, the efficacy of these drugs in the clinic needs to be established.

References

1. Clarke R, Cook KL, Hu R, Facey COB, Tavassoly I, Schwartz JL, et al. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. Cancer Res. 2012;72:1321–31.
2. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 2012;13:89–102.
3. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334:1081–6.
4. Pincus D, Chevalier MW, Aragón T, van Anken E, Vidal SE, El-Samad H, et al. BiP binding to the ER-Stress Sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. PLoS Biol. 2010;8:e1000415.
5. Hollien J. Evolution of the unfolded protein response. Biochim Biophys Acta Mol Cell Res. 2013;1833:2458–63.
6. Uemura A, Oku M, Mori K, Yoshida H. Unconventional splicing of XBP1 mRNA occurs in the cytoplasm during the mammalian unfolded protein response. J Cell Sci. 2009;122:2877–86.
7. Liou HC, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik-Le NJ, et al. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. Science. 1990;247:1581–4.
8. Iwakoshi NN, Lee A-H, Glimcher LH. The X-box binding protein-1 transcription factor is required for plasma cell differentiation and the unfolded protein response. Immunol Rev. 2003;194:29–38.
9. Lee A-H, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol. 2003;23:7448–59.
10. Tarn WY, Steitz JA. Pre-mRNA splicing: the discovery of a new spliceosome doubles the challenge. Trends Biochem Sci. 1997;22:132–7.
11. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature. 2002;415:92–6.

12. Wang Y, Xing P, Cui W, Wang W, Cui Y, Ying G, et al. Acute endoplasmic reticulum stress-independent unconventional splicing of XBP1 mRNA in the nucleus of mammalian cells. Int J Mol Sci. 2015;16:13302–21.
13. Gonzalez TN, Sidrauski C, Dörfler S, Walter P. Mechanism of non-spliceosomal mRNA splicing in the unfolded protein response pathway. EMBO J. 1999;18:3119–32.
14. Li H, Korennykh AV, Behrman SL, Walter P. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proc Natl Acad Sci. 2010;107:16113–8.
15. Tirosh B, Iwakoshi NN, Glimcher LH, Ploegh HL. Rapid turnover of unspliced Xbp-1 as a factor that modulates the unfolded protein response. J Biol Chem. 2006;281:5852–60.
16. Hollien J, Weissman JS. Decay of endoplasmic reticulum-localized mrnas during the unfolded protein response. Science. 2006;313:104–7.
17. Hassler J, Cao SS, Kaufman RJ. IRE1, a double-edged sword in pre-miRNA slicing and cell death. Dev Cell. 2012;23:921–3.
18. Bright MD, Itzhak DN, Wardell CP, Morgan GJ, Davies FE. Cleavage of BLOC1S1 mRNA by IRE1 is sequence specific, temporally separate from XBP1 splicing, and dispensable for cell viability under acute endoplasmic reticulum stress. Mol Cell Biol. 2015;35:2186–202.
19. Navon A, Gatushkin A, Zelcbuch L, Shteingart S, Farago M, Hadar R, et al. Direct proteasome binding and subsequent degradation of unspliced XBP-1 prevent its intracellular aggregation. FEBS Lett. 2010;584:67–73.
20. Uemura A, Taniguchi M, Matsuo Y, Oku M, Wakabayashi S, Yoshida H. UBC9 regulates the stability of XBP1, a key transcription factor controlling the ER stress response. Cell Struct Funct. 2013;38:67–79.
21. Chen H, Qi L. SUMO modification regulates the transcriptional activity of XBP1. Biochem J. 2010;429:95–102.
22. Wang F-M, Chen Y-J, Ouyang H-J. Regulation of unfolded protein response modulator XBP1s by acetylation and deacetylation. Biochem J. 2011;433:245–52.
23. Zambrano J, Ettayebi K, Maaty WS, Faunce NR, Bothner B, Hardy ME. Rotavirus infection activates the UPR but modulates its activity. Virol J. 2011;8:359.
24. Gupta A, Hossain MM, Miller N, Kerin M, Callagy G, Gupta S. NCOA3 coactivator is a transcriptional target of XBP1 and regulates PERK–eIF2α–ATF4 signalling in breast cancer. Oncogene. 2016;35:5860–71.
25. Tian P-G, Jiang Z-X, Li J-H, Zhou Z, Zhang Q-H. Spliced XBP1 promotes macrophage survival and autophagy by interacting with Beclin-1. Biochem Biophys Res Commun. 2015;463:518–23.
26. Margariti A, Li H, Chen T, Martin D, Vizcay-Barrena G, Alam S, et al. XBP1 mRNA splicing triggers an autophagic response in endothelial cells through BECLIN-1 transcriptional activation. J Biol Chem. 2013;288:859–72.
27. Gomez BP, Riggins RB, Shajahan AN, Klimach U, Wang A, Crawford AC, et al. Human X-Box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. FASEB J. 2007;21:4013–27.
28. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell. 2005;122:927–39.
29. Pehar M, Jonas MC, Hare TM, Puglielli L. SLC33A1/AT-1 protein regulates the induction of autophagy downstream of IRE1/XBP1 pathway. J Biol Chem. 2012;287:29921–30.
30. Zhou Y, Lee J, Reno CM, Sun C, Park SW, Chung J, et al. Regulation of glucose homeostasis through a XBP-1-FoxO1 interaction. Nat Med. 2011;17:356–65.
31. Cuevas EP, Eraso P, Mazón MJ, Santos V, Moreno-Bueno G, Cano A, et al. LOXL2 drives epithelial-mesenchymal transition via activation of IRE1-XBP1 signalling pathway. Sci Rep. 2017;7:44988.
32. Chen W, Zhou J, Wu K, Huang J, Ding Y, Yun E-J, et al. Targeting XBP1-mediated β-catenin expression associated with bladder cancer with newly synthetic Oridonin analogues. Oncotarget. 2016;7:56842–54.

33. Xia T, Tong S, Fan K, Zhai W, Fang B, Wang S-H, et al. XBP1 induces MMP-9 expression to promote proliferation and invasion in human esophageal squamous cell carcinoma. Am J Cancer Res. 2016;6:2031–40.
34. Zeng L, Xiao Q, Chen M, Margariti A, Martin D, Ivetic A, et al. Vascular endothelial cell growth-activated XBP1 splicing in endothelial cells is crucial for angiogenesis. Circulation. 2013;127:1712–22.
35. Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE, Blackwell TS. Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. J Biol Chem. 2011 Sep 2;286(35):30972–80. https://doi.org/10.1074/jbc.M110.181164. Epub 2011 Jul 11. https://www.ncbi.nlm.nih.gov/pubmed/21757695.
36. Zeng L, Zampetaki A, Margariti A, Pepe AE, Alam S, Martin D, et al. Sustained activation of XBP1 splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow. Proc Natl Acad Sci. 2009;106:8326–31.
37. Ding L, Yan J, Zhu J, Zhong H, Lu Q, Wang Z, et al. Ligand-independent activation of estrogen receptor alpha by XBP-1. Nucleic Acids Res. 2003;31:5266–74.
38. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Clarke R. NFκB Signaling is required for XBP1 (U and S) mediated effects on Antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol. 2015;35:379–90.
39. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1α pathway. Nature. 2014;508:103–7.
40. Hess DA, Strelau KM, Karki A, Jiang M, Azevedo-Pouly AC, Lee A-H, et al. MIST1 links secretion and stress as both target and regulator of the UPR. Mol Cell Biol. 2016;36:2931–44.
41. Yoshida H, Oku M, Suzuki M, Mori K. pXBP1(U) encoded in XBP1 pre-mRNA negatively regulates unfolded protein response activator pXBP1(S) in mammalian ER stress response. J Cell Biol. 2006;172:565–75.
42. Guo F, Lin EA, Liu P, Lin J, Liu C. XBP1U inhibits the XBP1S-mediated upregulation of the iNOS gene expression in mammalian ER stress response. Cell Signal. 2010;22:1818–28.
43. Benhamron S, Hadar R, Iwawaky T, So J-S, Lee A-H, Tirosh B. Regulated IRE1-dependent decay participates in curtailing immunoglobulin secretion from plasma cells. Eur J Immunol. 2014;44:867–76.
44. Zhao Y, Li X, Cai M-Y, Ma K, Yang J, Zhou J, et al. XBP-1u suppresses autophagy by promoting the degradation of FoxO1 in cancer cells. Cell Res. 2013;23:491–507.
45. Eijkelenboom A, Burgering BMT. FOXOs: signalling integrators for homeostasis maintenance. Nat Rev Mol Cell Biol. 2013;14:83–97.
46. Martin D, Li Y, Yang J, Wang G, Margariti A, Jiang Z, et al. Unspliced X-box-binding protein 1 (XBP1) protects endothelial cells from oxidative stress through interaction with histone deacetylase 3. J Biol Chem. 2014;289:30625–34.
47. Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, Okada T, et al. IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. Genes Dev. 2002;16:452–66.
48. Fujimoto T, Onda M, Nagai H, Nagahata T, Ogawa K, Emi M. Upregulation and overexpression of human X-box binding protein 1 (hXBP-1) gene in primary breast cancers. Breast Cancer. 2003;10:301–6.
49. Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, et al. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell. 2007;11:349–60.
50. Munshi NC, Hideshima T, Carrasco D, Shammas M, Auclair D, Davies F, et al. Identification of genes modulated in multiple myeloma using genetically identical twin samples. Blood. 2004;103:1799–806.
51. Shuda M, Kondoh N, Imazeki N, Tanaka K, Okada T, Mori K, et al. Activation of the ATF6, XBP1 and grp78 genes in human hepatocellular carcinoma: a possible involvement of the ER stress pathway in hepatocarcinogenesis. J Hepatol. 2003;38:605–14.

52. Shajahan AN, Riggins RB, Clarke R. The role of X-box binding protein-1 in tumorigenicity. Drug News Perspect. 2009;22:241–6.
53. Davies MPA, Barraclough DL, Stewart C, Joyce KA, Eccles RM, Barraclough R, et al. Expression and splicing of the unfolded protein response gene XBP-1 are significantly associated with clinical outcome of endocrine-treated breast cancer. Int J Cancer. 2008;123:85–8.
54. Ming J, Ruan S, Wang M, Ye D, Fan N, Meng Q, et al. A novel chemical, STF-083010, reverses tamoxifen-related drug resistance in breast cancer by inhibiting IRE1/XBP1. Oncotarget. 2015;6:40692–703.
55. Tozlu S, Girault I, Vacher S, Vendrell J, Andrieu C, Spyratos F, et al. Identification of novel genes that co-cluster with estrogen receptor alpha in breast tumor biopsy specimens, using a large-scale real-time reverse transcription-PCR approach. Endocr Relat Cancer. 2006;13(4):1109–20. https://www.ncbi.nlm.nih.gov/pubmed/17158757
56. Lacroix M, Leclercq G. About GATA3, HNF3A, and XBP1, three genes co-expressed with the oestrogen receptor alpha gene (ESR1) in breast cancer. Mol Cell Endocrinol. 2004;219(1–2): 1–7. https://www.ncbi.nlm.nih.gov/pubmed/15149721
57. Andres SA, Wittliff JL. Co-expression of genes with estrogen receptor-α and progesterone receptor in human breast carcinoma tissue. Horm Mol Biol Clin Investig. 2012;12:377–90.
58. Scriven P, Coulson S, Haines R, Balasubramanian S, Cross S, Wyld L. Activation and clinical significance of the unfolded protein response in breast cancer. Br J Cancer. 2009;101:1692–8.
59. Sengupta S, Sharma CGN, Jordan VC. Estrogen regulation of X-box binding protein-1 and its role in estrogen induced growth of breast and endometrial cancer cells. Horm Mol Biol Clin Investig. 2010;2:235–43.
60. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang H, et al. NFkappaB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol. 2015;35:379–90.
61. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature. 2014;508:103–7.
62. Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell. 2015;161:1527–38.
63. Bae J, Samur M, Munshi A, Hideshima T, Keskin D, Kimmelman A, et al. Heteroclitic XBP1 peptides evoke tumor-specific memory cytotoxic T lymphocytes against breast cancer, colon cancer, and pancreatic cancer cells. Oncoimmunology. 2014;3:e970914.
64. Jiang D, Niwa M, Koong AC. Targeting the IRE1α-XBP1 branch of the unfolded protein response in human diseases. Semin Cancer Biol. 2015;33:48–56.

Chapter 7
The Unfolded Protein Response in Triple-Negative Breast Cancer

Na Zhao, Fanglue Peng, and Xi Chen

Abstract Triple-negative breast cancer (TNBC) is the designation of the diverse and highly aggressive breast cancers that lack the expression of estrogen receptor, progesterone receptor, and HER2. Due to the lack of recognized molecular targets for therapy, chemotherapy remains the primary established treatment for TNBC. In TNBC, numerous exogenous and intrinsic factors cause accumulation of misfolded or unfolded proteins in the endoplasmic reticulum (ER) to disrupt cellular proteostasis in a condition termed ER stress. As an adaptation, cells activate a network of pathways, the unfolded protein response (UPR), to manage ER stress. Chronic stress, a risk factor in cancer initiation and progression, keeps the UPR engaged while its apoptotic function gets gradually attenuated. The UPR has been increasingly recognized to have crucial roles both in physiological contexts and tumor pathology. In this review, we summarize the factors that may contribute to the regulation of the UPR in TNBC and its impact on various aspects of tumor biology. In addition, we review recent progress on the pharmacological targeting of the UPR, which holds therapeutic potential for TNBC intervention.

Keywords UPR · ER stress · IRE1α · XBP1 · ATF6 · PERK · Triple-negative breast cancer

N. Zhao · F. Peng · X. Chen (✉)
Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA

Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA
e-mail: Xi.Chen@bcm.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_7

Abbreviations

ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
BC	Breast cancer
BiP	Binding immunoglobulin protein
BL	Basal-like
CHOP	C/EBP homologous protein
CREB3	cAMP responsive element binding protein 3
CREB3L	cAMP responsive element binding protein 3 like
DC	Dendritic cells
DR5	Death receptor 5
eIF2α	Eukaryotic translation initiation factor 2 alpha
EMT	Epithelial to mesenchymal transition
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated degradation
ERSE	Endoplasmic reticulum stress element
FAS	Fatty acid synthase
FFA	Free fatty acid
FOXO	Forkhead box O
GAAC	General amino acid control
GADD34	Growth arrest and DNA damage-inducible protein 34
GCN2	General control nonderepressible 2
GEF	Guanine nucleotide exchange factor
HIF1α	Hypoxia-inducible factor 1α
HRI	Heme-regulated eIF2α kinase
IM	Immunomodulatory
IRE1α	Inositol-requiring enzyme 1α
ISR	Integrated stress response
JNK	JUN N-terminal kinase
LAR	Luminal androgen receptor
M	Mesenchymal
MEF	Mouse embryonic fibroblasts
MSL	Mesenchymal stem-like
NRF2	Nuclear factor erythroid 2-related factor 2
PDAC	Pancreatic ductal adenocarcinoma
PDX	Patient-derived xenograft
PERK	Protein kinase RNA (PKR)-like ER kinase
PHD3	Prolyl-4-hydroxylase domain 3
PI3K	Phosphoinositide 3-kinase
PR	Progesterone receptor
RIDD	Regulated IRE1α-dependent decay
ROS	Reactive oxygen species
SERCA	Sarcoplasmic/endoplasmic reticulum calcium ATPase
SREBP	Sterol regulatory element binding protein

TCA	Tricarboxylic acid cycle
TCGA	The cancer genome atlas
TNBC	Triple-negative breast cancer
TRAF2	TNF-receptor-associated factor 2
uORF	Upstream open reading frame
UPR	Unfolded protein response
UPRE	Unfolded protein response element
VHL	von Hippel-Lindau
XBP1	X-box binding protein 1

Introduction

During tumor initiation and progression, cancer cells are subjected to various forms of intra- and extra-cellular stresses, resulting in significant proteostatic perturbations [1–4]. Adaptation to these cytotoxic stresses from early on is crucial for the selection and survival of viable cancer cell lineages. Recent studies have shown that the unfolded protein response (UPR) is activated in many human cancers and plays important roles in tumor initiation and progression [3–10]. The UPR is an evolutionarily conserved, intricate cellular homeostatic network that has developed to enable cells to adapt to fluctuations in protein demand and folding capacity, while protecting them against the ensuing stress in the endoplasmic reticulum (ER) caused by the accumulation of unfolded or misfolded proteins. These cellular adaptations may include expansion of the ER to support the higher protein demand, downregulation of global translation to relieve the overall protein load, and selective upregulation of chaperones and enzymes involved in post-translational processes to increase folding efficiency. The UPR can also promote ER-associated degradation (ERAD) to facilitate the elimination of proteotoxic elements and initiate complete cellular programs such as autophagy or even apoptosis if prior attempts are unsuccessful. This latter pathway emphasizes the priority of the organism over the cell and elevates the UPR from the cellular level to the systemic level. To execute such complex and vital processes, the UPR is required to evaluate and respond correctly to the extent, context, and timing of these perturbations, and to properly distinguish between acute and chronic conditions. This is accomplished through an intricate network of the three individual arms of the UPR, their crosstalk, feedback loops, and their branching out to other networks of cellular pathways and programs. These features render the UPR essential in the understanding of cancer while also offering a number of unique and potentially global ways of interventions. There are extensive reviews on the UPR and its significance in cancer with relevant therapeutic potentials [3, 4, 11]. In this review, we focus on the characteristics of the UPR and its implications in triple-negative breast cancer (TNBC).

Upon amplified folding demand, the UPR is triggered by the simultaneous dissociation of the ATPase chaperone, binding immunoglobulin protein (BiP, encoded by *HSPA5*), from all three transmembrane sensor-actuator proteins in the ER membrane [12–16]. Upon activation, these enzymes, namely, inositol-requiring enzyme

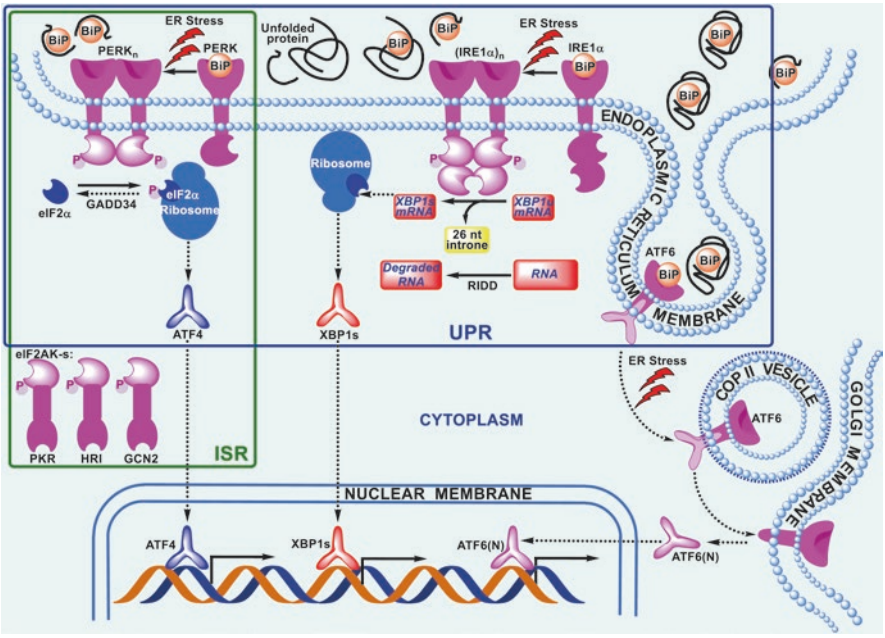

Fig. 7.1 The unfolded protein response (UPR) signaling pathways. Upon accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), the UPR is triggered by the simultaneous dissociation of binding immunoglobulin protein (BiP) from three transmembrane proteins in the ER membrane, which are protein kinase RNA (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6). Activated stress sensors send their distinct signals from the ER lumen to their respective downstream arms to enable the cells to adapt to proteostatic perturbations. In the PERK branch, activated PERK dimerizes, autophosphorylates, and then phosphorylates the eukaryotic translation initiation factor 2α (eIF2α) to attenuate cap-dependent protein translation while increasing the translation of activating transcription factor 4 (ATF4). Three other kinases, namely, double-stranded RNA-dependent protein kinase (PKR), heme-regulated eIF2α kinase (HRI), and general control nonderepressible2 (GCN2) converge with PERK on the phosphorylation of eIF2α. These four eIF2α kinases constitute the integrated stress response (ISR) pathway. Growth arrest and DNA damage-inducible protein 34 (GADD34) recruits protein phosphatase to dephosphorylate eIF2α and restore global mRNA translation. In the IRE1α branch, IRE1α oligomerizes and *trans*-autophosphorylates to activate its cytosolic RNase domain under ER stress. Activated IRE1α excises 26 nucleotides from the unspliced X-box binding protein 1 (XBP1) mRNA (*XBP1u*), resulting in a frameshift to produce the spliced *XBP1* (*XBP1s*), which encodes the active transcription factor XBP1s. Prolonged hyperactivation of IRE1α further degrades many mRNAs and non-coding RNAs in a process called regulated IRE1α-dependent decay (RIDD). In the ATF6 branch, the disassociation of BiP releases ATF6 for translocation from the ER membrane to the Golgi apparatus, where ATF6 undergoes regulated intramembrane proteolysis that turns its cytosolic N-terminal into a mature transcription factor ATF6 (N)

1 (IRE1α, encoded by *ERN1*), protein kinase RNA (PKR)-like ER kinase (PERK, encoded by *EIF2AK3*) and activating transcription factor 6 (ATF6), send their distinct signals from the ER lumen to their respective downstream arms to enable the cells to adapt to these proteostatic perturbations (Fig. 7.1).

IRE1α

The bifunctional kinase-RNase IRE1α is the most ancient and conserved member of the mammalian sensory triad of the UPR [17–19]. Under ER stress, IRE1α oligomerizes and *trans*-autophosphorylates to activate its cytosolic RNase domain [20, 21]. This activated form of IRE1α then excises 26 nucleotides from the unspliced X-box binding protein 1 (XBP1) mRNA (*XBP1u*), resulting in a frameshift to produce the mature, spliced *XBP1* (*XBP1s*) [22–25]. This unique cytosolic splicing mechanism, paired with an instant translation by co-localized ribosomes, enables a rapid XBP1 isoform switching in response to acute perturbations in ER proteostasis [26]. Phosphatases, especially ER-localized protein phosphatase PP2Ce (encoded by *PPM1L*), can dephosphorylate IRE1α and influence this process [27]. Importantly, PP2Ce deficiency can trigger pathological stress signaling in postpartum mammary glands [28]. *XBP1u* is also translated into a short-lived cytosolic protein. However, it is quickly degraded by the proteasome and acts as a dominant-negative form of XBP1s. Transcription factor XBP1s enters the nucleus, where it starts the canonical transcriptional stress response through the cis-acting UPR element (UPRE) and ER stress element (ERSE) [26]. XBP1s also participates in a number of context-dependent processes (vide infra). Nevertheless, this acute phase of activation of IRE1α RNase may be exhausted over time under persistent stress due to the clocking effect of its autophosphorylation by the kinase domain to temporarily desensitize its RNase [29, 30].

Subsequently, as stress persists, prolonged hyperactivation of IRE1α further promotes its aggregation, which enhances the functions of both its RNase and kinase. The RNase becomes increasingly promiscuous and degrades hundreds of RNAs, including many mRNAs, rRNAs and some non-coding RNAs, as well as its own mRNA, which establishes another negative feedback loop. This process is called regulated IRE1α-dependent decay (RIDD) [31]. RIDD further attenuates translation through decreasing the quantity of mRNAs and the integrity of the ribosomes. A thorough classification of RIDD substrates in the context of their sequence specificity, localization, and function has been reviewed elsewhere [32]. A model was also proposed how, with extended stress, RIDD becomes prevalent and contributes to the shift from cell survival to apoptosis [32]. However, under normal or low stress conditions, the physiological relevance of RIDD is not completely understood and warrants further investigation. Additionally, the aggregation of IRE1α positions its kinase domains to form a platform for protein docking, particularly for the adaptor protein TNF-receptor-associated factor 2 (TRAF2), which relays signal to the JUN N-terminal kinase (JNK) pathway to directly initiate autophagy and ultimately again, apoptosis [33].

PERK

Another arm of the UPR is initiated by PERK, which becomes activated via oligomerization and autophosphorylation under proteotoxic ER stress [34]. However, unlike IRE1α, PERK also phosphorylates several cytosolic substrates, most significantly, the α subunit of eukaryotic translation initiation factor 2 (eIF2α). Phosphorylation of eIF2α dampens cap-dependent protein translation and attenuates the overall protein burden on the ER [35]. Meanwhile, some genes with unique short upstream open reading frames (uORFs) in their mRNA are translationally induced upon eIF2α phosphorylation, the most prominent of which is activating transcription factor 4 (ATF4) [36]. Thus, in addition to translational regulation, PERK also invokes transcriptional regulation to merge the UPR with the integrated stress response (ISR), as it is one of the eIF2α kinase tetrad [37]. A negative feedback loop exists along this pathway that is mediated by ER-localized growth arrest and DNA damage-inducible protein 34 (GADD34, encoded by *PPP1R15A*), which recruits protein phosphatase 1C to dephosphorylate eIF2α and restore global mRNA translation [38]. GADD34 is transcriptionally activated by ATF4, and translationally upregulated by cap-independent translation upon PERK activation due to the existence of 5′ uORF in its mRNA [39, 40]. ATF4 then directly modulates autophagy and, under runaway stress conditions, apoptosis via transcription factor C/EBP homologous protein (CHOP, encoded by *DDIT3*) [41]. However, just before an ultimate apoptotic termination, a final temporary safeguarding delay halts CHOP translation via miR-211, also one of the ATF4 targets [42]. These time-sensitive responses by GADD34 and miR-211 consequently enable the PERK arm, similar to the IRE1α arm, to distinguish among varying stress levels and between acute and chronic stress. Additionally, PERK also initiates responses to oxidative stress and is involved in stress-related metabolic reprogramming through the phosphorylation of transcription factors nuclear factor erythroid 2-related factor 2 (NRF2) and forkhead box O (FOXO), respectively [43, 44].

ATF6

The third arm of the UPR triad is initiated by ATF6, which, under proteotoxic ER stress, translocates from the ER membrane to the Golgi where it undergoes two site specific intramembrane proteolytic cleavages that turn its cytosolic N-terminal into a mature transcription factor [45]. ATF6 has two mammalian isoforms, namely, ATF6α and ATF6β, and they mature in parallel. The latter is considered a repressor of the former, which is the active transcription factor [46]. However, unlike the IRE1α and PERK arms, maturation of ATF6 is irreversible and unidirectional with no transcriptional or translational amplification and no direct involvement of a kinase-phosphatase feedback loop. This implies a simplified role of this arm in the time-sensitive decisions of the UPR. Indeed, the general view on the function of ATF6 is to balance, fine-tune, and provide a steady stream of supply to the already

established responses from the other two arms [26, 47]. However, mice deficient in both isoforms (*ATF6α* and *ATF6β*) are embryonic lethal, while the single knockout mice of either isoforms develop normally [48]. This observation suggests functional redundancy of the two isoforms in mouse development. The canonical role of ATF6 is executed in the nucleus on ERSE, and its targets include the genes of *BiP*, *CHOP*, and *XBP1u*, which merge this arm with the other two arms at the transcriptional level [22, 48–50].

Several other ER stress sensors are activated in similar regulated intramembrane proteolytic mechanisms as those of ATF6, including cAMP responsive element binding protein 3 (CREB3), and cAMP responsive element binding protein 3 like 1–4 (CREB3L1, CREB3L2, CREB3L3, CREB3L4) [51–54]. They have been reported to have important functions in astrocytes, osteoblasts, and chondrocytes. However, it is not known whether these sensors play any roles in breast cancer.

Crosstalk Among the Three UPR Arms

The three arms of the UPR are not isolated from each other but they are rather intertwined [55]. The PERK and IRE1α arms exert opposing effects on the expression of death receptor 5 (*DR5*) mRNA, therefore *DR5* becomes their intermediator on the determination of cell fate [56]. There is also direct crosstalk between the arms. As mentioned before, the ATF6 arm participates in a dialogue with the other arms of the UPR by transactivating *CHOP* and *XBP1u* [22, 50]. The ATF6 arm is not alone. Indeed, the PERK and IRE1α arms are complex and widespread enough to have multiple overlaps and intersections. A single-cell imaging technique was used to dissect the dynamics of these arms upon cell survival response [57]. While finding these interactions is particularly challenging, due to their complexity and context-dependence, some of the crosstalk has been uncovered. For example, coactivator NCOA3, an XBP1 transcriptional target, can regulate PERK–eIF2α–ATF4 signaling in breast cancer [58]. Moreover, *IRE1α* mRNA and *XBP1* splicing are induced by ATF4 under acute ER stress [59]. The PERK-ATF4 pathway also promotes the synthesis of ATF6 and its translocation from the ER to the Golgi [60]. Thus, each arm can influence the other two arms.

Physiological Role of the UPR in Mammary Gland Development

Cells that have a high secretory demand are particularly reliant on a well-developed and properly functional ER and are thus more susceptible to disturbances in the ER. For example, genetic ablation of *XBP1* results in dysfunction in highly secretory plasma cells, pancreatic acinar cells, and Paneth cells [61]. The mammary gland is a highly secretory tissue that provides newborns with milk protein, lipids,

and essential supplements. During pregnancy and lactation, hormonal changes in prolactin, placental lactogens, and progesterone induce extensive biosynthetic and secretory activity [62]. Not surprisingly, the UPR plays a crucial role in mammary gland development and function.

Mammary epithelial stem/progenitor cells with *BiP* deletion fail to generate repopulated mammary glands in de-epithelialized recipient mice, indicating that BiP is required for pubertal mammary gland development [63]. However, the general lethal effect of *BiP* knockout should not be ignored in this context. During pregnancy, the mRNA expression of *Atf4* and *Xbp1* dramatically increases in mouse mammary glands. Deletion of *Xbp1* in GFAP-expressing mammary epithelium ($Xbp1^{f/f}$; GFAP-Cre) not only impairs mammary gland branching and terminal end bud formation during the adult virgin period, but also dramatically inhibits mammary epithelial proliferation and differentiation during the lactation period [64]. The expression levels of both prolactin receptor and *ErbB4* are decreased in *Xbp1*-deficient mammary epithelium through unknown mechanisms. This results in reduced synthesis of milk protein (α/β-casein and whey acidic protein), leading to poor pup growth and postnatal mortality [64]. In another study, *Xbp1* deletion in ovine β-lactoglobulin-expressing mammary epithelial cells ($Xbp1^{f/f}$; BLG-Cre) caused significant reduction of the mammary epithelium during lactation [65]. Mice with *Perk* knockout in mammary epithelial cells produce milk with altered lipid composition, leading to protracted pup growth. In this context, the PERK/eIF2α signaling pathway enables the functional maturation of milk-secreting mammary epithelial cells by sustaining the expression of lipogenic enzymes Fasn, Acl, and Scd1 [66].

Role of the UPR in TNBC

In addition to having crucial functions under physiological conditions, the UPR is involved in various pathological conditions, including cancer [3, 4, 11, 67]. UPR has been extensively studied in human breast cancer. XBP1 is a classical estrogen receptor target [68, 69]. Clarke and colleagues' pioneering studies elucidate the essential roles of XBP1 in endocrine resistance [70–78]. XBP1 mRNA correlates with tamoxifen responsiveness of estrogen receptor-positive breast cancer patients. Silencing of *XBP1* sensitizes the endocrine therapy-resistant breast cancer cells to antiestrogens, while overexpression of XBP1s confers the estrogen receptor-positive tumors resistance to tamoxifen [74]. Recent studies also revealed unexpected function of UPR in TNBC [5]. TNBC is a heterogenous subtype of breast cancer characterized by the absence of expression of estrogen receptor, progesterone receptor (PR), and HER2—signaling receptors that are known to fuel the majority of breast cancers. TNBC is very aggressive and more likely to relapse and metastasize than the other breast cancer subtypes [79, 80]. To dissect TNBC-specific tumor heterogeneity, Pietenpol et al. classified TNBC into six subtypes on the basis of gene-expression profiles: two basal-like (BL)-related subgroups (BL1 and BL2), two mesenchymal-related subgroups (mesenchymal (M) and mesenchymal stem-like (MSL)), one immunomodulatory (IM) subgroup, and one luminal androgen

receptor (LAR) subgroup [81]. The overwhelming majority of TNBCs were classified as basal-like (50~75%) [82].

Typically, most TNBCs have mutations and deletions in *TP53*, while a significant subset arises in the context of germline or somatic mutations in *BRCA1* or *BRCA2* [83, 84]. Mutations in other druggable oncogenes, such as the protein-tyrosine kinases *PIK3CA* and *BRAF*, are not prevalent in TNBC [83, 84]; therefore, little progress has been made in treating this disease. The development of effective therapies is urgently needed to treat TNBC especially its metastasis and relapse. Recent studies have revealed the UPR as an important mechanism that promotes TNBC progression and therapy resistance [5]. Activation of many components of the UPR is also associated with patient outcomes (Table 7.1) [5, 70, 85–89]. Several

Table 7.1 Expression status and prognosis value of the UPR components in human breast cancer

Breast cancer subtypes	UPR components	Status[a]	Detection method	Prognosis	Reference
TNBC	BiP	Increased	IHC	Correlated with more lymph node metastasis and poor overall survival	[85]
Mixed		Unknown	IHC	Correlated with worse disease-free survival	[86]
ERα⁺, TN, and HER2-amplified BC		Increased	IHC	NA	[70]
TNBC	p-eIF2α	Increased	IHC	Correlated with better disease-free survival	[87]
TNBC	XBP1 signature	Unknown	Microarray	Correlated with shorter relapse-free survival	[5]
Mixed	*XBP1s/XBP1u*	Unknown	qPCR	Correlated with shorter relapse-free survival	[88]
ERα⁺ BC		Unknown	qPCR	Correlated with shorter relapse-free survival	[88]
Mixed	*XBP1u*	Unknown	qPCR	Correlated with better relapse-free survival	[88]
ERα⁺ BC		Unknown	qPCR	Correlated with better relapse-free survival	[88]
Mixed	CHOP	Unknown	IHC	Correlated with prolonged disease-free survival	[86]
ERα⁺ BC	UPR gene signature	Unknown	Microarray	Correlated with poor disease-free and overall survival	[89]

TNBC triple-negative breast cancer; *ERa+*, estrogen receptor a-positive; *TN* triple-negative; *BC* breast cancer; *BiP* binding immunoglobulin protein (encoded by HSPA5); *p-eIF2α* phosphorylated eukaryotic translation initiation factor 2 alpha; *XBP1* X-box binding protein 1; *XBP1s* spliced XBP1; *XBP1u* unspliced XBP1; *XBP1s/XBP1u* XBP1 splicing ratio; *CHOP* C/EBP homologous protein (encoded by DDIT3); *UPR* unfolded protein response; *IHC* immunohistochemistry; *qPCR* real-time quantitative PCR; *NA* not applicable

[a]Status indicates expression level in tumor compared to nontumor tissues

fundamental questions remain to be answered to pharmacologically target the UPR and improve clinical outcomes of patients with TNBC. This section discusses the function and mechanistic insights of UPR in TNBC.

Pathological Stimuli for UPR Activation

Numerous physiological and pathological stimuli disturb the ER protein folding environment and trigger ER stress (Fig. 7.2) [2]. Although we usually only encounter these altered cells of diverse pathologies, the underlying stress and its effects on their UPR can serve as Ariadne's thread to reveal the process of malignant transformation. Uncovering the underlying causes of these stresses in cancer, deciphering the interactions between these stresses and the UPR, and deconvoluting the dynamics that leads to cancer initiation and progression, will help us understand how cancer cells are eventually able to conquer the UPR and bias it towards decisions that lead to cell survival. In the context of TNBC, the questions to ask are—what are these stresses, and what is the underlying mechanism for the enhanced UPR activation in TNBC? To supplement the rather scarce data concerning the involvement of the UPR in TNBC, information from cancers of other tissues will also be considered.

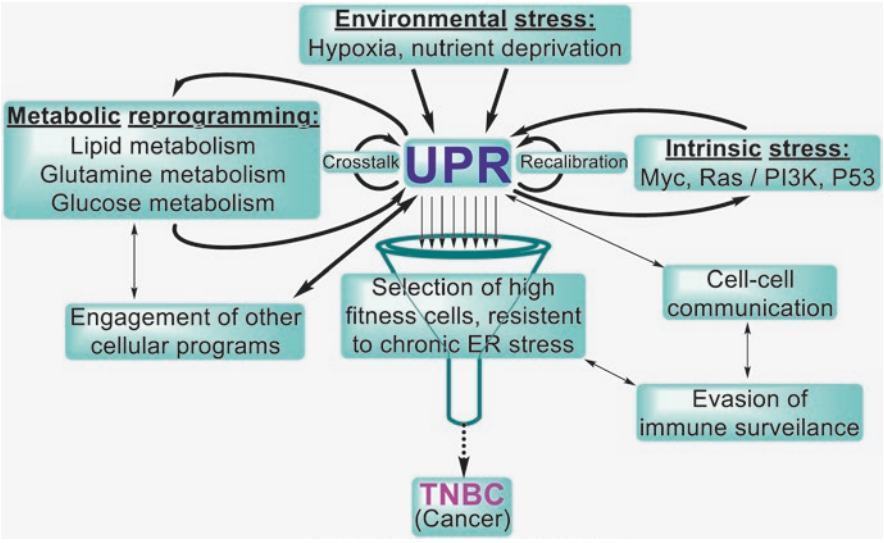

Fig. 7.2 The unfolded protein response (UPR) in triple-negative breast cancer (TNBC). Various environmental and intrinsic factors during tumorigenesis disturb ER homeostasis and activate the UPR. Extended ER stress usually kills the cells but it may also lead to the selection of cells with high fitness that have an altered UPR. Extended activation of the UPR advances the emergence of the hallmarks of TNBC

Environmental Stress

Various environmental and intrinsic factors during tumorigenesis disturb ER homeostasis. For example, synthesis, folding, and post-translational processing of proteins require abundant energy supply. However, the inadequate nutrient supply in the tumor microenvironment compromises these processes and instigates the accumulation of unfolded proteins in the ER and triggers ER stress. As solid tumors grow, their demand for oxygen and nutrient eventually exceeds the capacity of the existing blood supply [90]. Although many tumors induce angiogenesis as an adaptation mechanism, the center of the tumor is still unavoidably challenged by the lack of oxygen and nutrition.

Hypoxia

Hypoxia is a common feature of most solid tumors. Hypoxia induced signaling cascade plays pivotal roles in tumor biology, including promotion of tumor angiogenesis and metastasis, metabolic alteration, immune suppression, heightened genomic instability, and consequently, therapy resistance [90, 91]. Severe hypoxia affects cysteinyl oxidation to form disulfide bonds and disrupts disulfide isomerization of proteins during post-translational folding and modification, leading to protein misfolding and ER stress [92]. As expected, heightened XBP1 splicing, eIF2α phosphorylation, ATF4 and CHOP protein expression are observed under extreme hypoxia [93–96]. Hypoxia also inhibits the degradation of the ATF4 protein [97]. Under normoxia, prolyl-4-hydroxylase domain 3 (PHD3) interacts with ATF4 and hydroxylates the proline residues adjacent to its zipper domain to target ATF4 for von Hippel-Lindau (VHL) ubiquitin ligase-independent proteasomal degradation. Hypoxia suppresses the hydroxylation-dependent degradation and stabilizes the ATF4 protein [97]. Furthermore, an analysis of the cancer genome atlas (TCGA) datasets showed that activation of the hypoxia-inducible factor 1α (HIF1α) signaling network is a key regulatory feature in TNBC [83]. Genetic deletion of XBP1 was shown to suppress the transcriptional activity of HIF1α, thus blocking TNBC tumor progression [5]. Taken together, these studies underline the importance of hypoxia as a UPR stressor in the tumor microenvironment and indicate that the molecular connection between hypoxia and UPR is critical to sustain TNBC progression.

Nutrient Deprivation

Abnormal vascularization in solid tumors leads to short supply of nutrients that further mobilizes the UPR. The surviving cancer cells adapt to the ensuing chronic shortages through engaging the UPR and ISR to reprogram the cellular metabolism [98–100]. The ISR is mediated by four kinases, PERK, double-stranded RNA-dependent protein kinase (PKR, encoded by *EIF2AK2*), heme-regulated eIF2α

kinase (HRI, encoded by *EIF2AK1*), and general control nonderepressible 2 (GCN2, encoded by *EIF2AK4*). These four sensors converge on the phosphorylation of eIF2α. Amino acid depletion activates GCN2 upon its sensing of uncharged tRNAs [101]. GCN2 phosphorylates eIF2α and induces GCN4 and ATF4 translation. GCN4 is the primary regulator in response to amino acid starvation, termed general amino acid control (GAAC) [102]. Acting as a transcription factor, GCN4 activates several genes required for amino acid synthesis. It also downregulates key components of the lipid biosynthesis such as fatty acid synthase (FAS). Activated ATF4 transcribes genes encoding amino acid transporters and synthesis enzymes, therefore augmenting amino acid metabolism and adaptation to amino acid deprivation [98].

Glucose insufficiency triggers ER stress through multiple mechanisms. Glucose metabolism provides tumor cells with ATP and building blocks for biosynthesis. This incapacitation alone would be a cause for alarm. Moreover, glucose is a donor for asparagine (N)-linked glycosylation, which is an important form of protein post-translational modifications synthesized inside the ER. Shortage of glucose results in improper protein glycosylation and protein misfolding [100]. Tunicamycin, an inhibitor of N-linked protein glycosylation, is a potent ER stress inducer. Moreover, under glucose insufficiency, the reduced energy supply dampens the activity of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), leading to disturbed Ca^{2+} homeostasis in the ER and inducing ER stress [103]. Thapsigargin, an inhibitor of SERCA, is another potent ER stress inducer [104].

Tumor cells invoke all three arms of the UPR in response to glucose deficiency, which induces eIF2α phosphorylation, ATF6 proteolytic activation, and XBP1 splicing [105–107]. Intriguingly, different upstream eIF2α kinases play distinct roles in glucose-deficient cells. Whereas *Perk*$^{-/-}$ and *Pkr*$^{-/-}$ mouse embryonic fibroblasts (MEFs) are more susceptible to the proapoptotic effect of glucose deprivation, the survival of *Gcn2*$^{-/-}$ MEFs is better than their wild-type counterparts [107]. This implies a cytoprotective role of PERK and PKR and a proapoptotic role of GCN2 in response to glucose deficiency. The proapoptotic function of GCN2 in this context is rather unexpected, since it is required for the adaptation of cells to amino acid deprivation. The underlying mechanism is unclear. Taken together, glucose deprivation and amino acid deficiency convene disparate pathways that converge on eIF2α phosphorylation with distinct biological outcomes.

Cell Intrinsic Factors

In the absence of external stressors, TNBC exhibits higher UPR activity than luminal breast cancers. This was demonstrated by a more dilated ER morphology, higher ratio of XBP1 splicing, and increased phosphorylation of PERK and eIF2α [5]. The elevated basal level UPR activation suggests the existence of unrecognized intracellular UPR stressors. One possible underlying mechanism could be attributed to the

secretory nature of TNBC tumors, especially during epithelial to mesenchymal transition (EMT). Cells undergoing EMT synthesize and secrete large quantities of extracellular matrix proteins and display an extensive branched ER morphology [6]. Activated PERK/eIF2α signaling is required for the invasive phenotype of cells undergoing EMT. Treatment of these cells with PERK inhibitor 3-fluoro-GSK2606414 reduces their ability to form tumorspheres and migrate in vitro. In addition, pretreatment of metastatic breast cancer cells with 3-fluoro-GSK2606414 significantly impaired their metastatic capacity in vivo [6]. Similarly, a recent study showed that the mesenchymal subpopulations of pancreatic ductal adenocarcinoma (PDAC) exhibit elevated protein turnover rates and enlarged ER. Genetic perturbation of *IRE1α* resulted in tumor regression and prolonged survival of tumor-bearing mice [108]. These studies indicate that an active UPR augments the fitness of mesenchymal-like cancer cells, and the elevated UPR might be a common feature of mesenchymal-like tumors.

Loss of function of tumor suppressors and hyperactivation of oncogenes are hallmarks of cancer cells. These genetic events increase protein synthesis to meet the increased metabolic demand during tumorigenesis. In addition, proliferating cells require ER expansion for division and allocation to daughter cells [109]. Extensive studies have demonstrated that deregulated genetic alterations in cancer cells could modulate the UPR signaling to enhance the processing capability and biogenesis of the ER.

MYC

The MYC family of proto-oncogenes, consisting of *MYC*, *MYCN,* and *MYCL*, are essential regulators of cell growth, proliferation, apoptosis, and metabolism. The aberrant activation of MYC is one of the most common features in human cancers. Hyperactivation of MYC is manifest in massive transcriptional and translational upregulations [110, 111]. MYC-induced escalation of protein synthesis alters the proportions of protein subunits in macromolecular complexes, leading to the buildup of unfolded and misfolded proteins that cause extensive proteotoxic stress. Koumenis and colleagues showed in lymphomas that the PERK branch of the UPR was activated indirectly by the elevated ER protein load resulted from MYC-driven protein synthesis and ribosome biogenesis. Activated PERK/eIF2α signaling induces cytoprotective autophagy, thus promoting cell survival and tumor formation [112]. The MYC-induced global elevation of protein synthesis also activates the IRE1α/XBP1 pathway [112]. However, the function of IRE1α/XBP1 in MYC-driven tumors remains elusive. The crosstalk between the PERK and IRE1α/XBP1 pathways and their possible redundancy or reciprocity is also largely unknown in MYC-driven tumors. As several studies have demonstrated MYC as one of the key features in TNBC [83, 113], it is plausible that MYC might contribute to the elevated activity of the UPR in TNBC.

RAS and Phosphoinositide 3-Kinase (PI3K)

RAS family proteins are master regulators of signaling pathways that emanate from cell surface receptors. Three members of the RAS family, HRAS, KRAS, and NRAS, are highly homologous, 85% of the amino acid sequences are identical [114]. Although they are known to act in very similar ways, their subtle differences are emerging. The components of the RAS/RAF/MAPK signaling cascade are rarely mutated but frequently amplified in basal-like breast cancer (amplification of *KRAS* and *BRAF* are 32% and 30%, respectively) [83]. One of the downstream signaling of RAS is the PI3K/AKT pathway, which regulates most of the hallmarks of cancer, including cell cycle, survival, metabolism, motility, and genomic instability [115]. Deep sequencing of patient tumors showed that *PIK3CA* is the second most commonly mutated gene (9%) in basal-like breast cancers [83]. Loss of *PTEN* or *INPP4B* also occurs in basal-like breast tumors [83]. Although the molecular connection between the RAS/RAF/MAPK, PI3K/AKT pathways and the UPR signaling in breast cancer is unclear, stable transduction of $HRAS^{G12V}$ but not of $BRAF^{V600E}$ into primary human melanocytes was shown to induce the expansion of ER content and expression of BiP, ATF4 and XBP1 (both unspliced and spliced forms). Activated UPR enhances $HRAS^{G12V}$-induced premature senescence through unknown mechanisms, thus posing a barrier for oncogenic transformation. The main alternative signaling cascade activated by HRAS besides BRAF is the PI3K/AKT pathway. Treatment of cells with the PI3K antagonist LY294002 or introduction of a dominant-negative AKT mutant ($AKT^{S73A/T308A}$) abolished $HRAS^{G12V}$-induced UPR activation and senescence. Notably, stable transduction of $NRAS^{Q61R}$ into primary human melanocytes induces lower levels of BiP and ATF4 compared to that of $HRAS^{G12V}$. The impaired induction of the UPR is correlated with a decreased activation of AKT by $NRAS^{Q61R}$, which is consistent with previous findings that NRAS is less effective in activating PI3K/AKT pathway than HRAS [116]. These data indicate a potential role of the RAS and PI3K/AKT signaling in the activation of the UPR in TNBC.

TP53

The p53 tumor suppressor regulates numerous cellular stress responses, including DNA repair, cell cycle arrest, and apoptosis [117]. *TP53* is the most commonly mutated gene in basal-like breast cancers (84%), whereas it is only mutated in 12% of luminal A and 32% of luminal B breast cancers. *TP53* is also one of the most frequently deleted genes in all subtypes of breast cancer [83]. Wild-type p53 has been shown to suppress IRE1α expression by interacting with and enhancing the activity of synoviolin 1 (encoded by *SYVN1*), an ER transmembrane E3 ubiquitin ligase that stimulates IRE1α degradation. Loss of p53 stimulates IRE1α-XBP1 signaling by abolishing p53-dependent association of IRE1α with synoviolin 1. Meanwhile, mutant p53 proteins ($p53^{G245S}$, $p53^{R248W}$, $p53^{R249S}$, and $p53^{R273H}$) that lack DNA-binding and transactivating functions do not regulate IRE1α expression. The

IRE1α RNase inhibitor STF-083010 selectively induces cell death and suppresses tumor growth in p53-deficient cancer cell lines but not in cell lines with wild-type p53 [118]. Thus, prevalent *TP53* deletion might be a potential mechanism for the elevated IRE1α-XBP1 activity in TNBC. It will be interesting to further investigate the amino acids mediating p53's interaction with synoviolin 1, examine their mutations in TNBC and test whether these mutations correlate with the activation of the IRE1α-XBP1 arm of the UPR.

Interestingly, PERK was reported to regulate p53 stability. Under ER stress, the ensuing ribosomal stress caused by translational suppression due to eIF2α phosphorylation liberates ribosomal proteins, some of which bind and sequester ubiquitin ligase MDM2, a major factor promoting p53 degradation. The succeeding accumulation of p53 was shown to lead to cell cycle arrest at the G_1/S restriction point [119]. This action amplifies the influence of PERK on ER stress-dependent cell cycle arrest in addition to the translational suppression of cyclin D1 [120].

UPR and the Hallmarks of TNBC

Activation of all three arms of the UPR has been widely documented in various types of cancer. Involvement of the UPR is usually a tell-tale sign of underlying stress. Chronic stress is always a risk factor to cells. As a response, extended engagement of the UPR ultimately kills the cell if the stress persists. Alternatively, it may also lead to the selection of cells that have adapted to these new conditions. This might eventually result in cell lineages with high fitness that have an altered UPR. Increasing evidence links the UPR to hallmarks of cancer, including survival, resistance to cell death, unchecked proliferation, metabolic reprogramming, metastasis, and remodeling of microenvironment.

UPR in Tumor Survival

The extent of UPR activation is essential for determining the fate of cancer cells. Both hyperactivation and insufficient induction of the UPR are detrimental to cells [121, 122]. Activation of PERK/eIF2α signaling mediates adaptation of cells to a hypoxic microenvironment. Deletion of *Perk* or introduction of phosphorylation-incompetent Ser51Ala (S51A) mutation to eIF2α was shown to promote cell death during hypoxia in MEFs [93, 96]. Furthermore, ATF4 and CHOP, two downstream transcription factors of PERK signaling, facilitate autophagy by activating essential autophagy genes including *MAP 1LC3B*, *ATG5*, *ATG6*, and *ATG8*, thus sustaining cell survival under hypoxia or during extracellular matrix detachment [123, 124]. PERK/eIF2α signaling has also been shown to promote the survival of radiation-resistant hypoxic cells through the induction of glutathione synthesis and attenuation of the reactive oxygen species (ROS) generation during cyclic acute hypoxia [95].

The IRE1α/XBP1 pathway is also required for the survival of cells under hypoxic conditions [94]. *XBP1* is constitutively spliced in TNBC, and the elevated expression of an XBP1 signature correlates with poor relapse-free survival of TNBC patients. Silencing of XBP1 inhibits tumor growth, blocks tumor relapse following chemotherapeutic treatment, and reduces the $CD44^{high}CD24^{low}$ population. Mechanistically, XBP1s promotes TNBC tumorigenicity by forming a transcriptional complex with HIF1α that regulates HIF1α targets via recruitment of RNA polymerase II [5].

Conversely, unmitigated UPR activation induces cell apoptosis [122]. The mechanism governing apoptosis initiation under prolonged ER stress is beginning to emerge. Recently, death receptor 5 (DR5, encoded by *TNFRSF10B*), a crucial protein for activating caspase 8, was proposed to integrate opposing UPR signals to control ER stress-induced apoptosis: whereas CHOP activity induces DR5 transcription, IRE1α catalyzes DR5 mRNA decay. As ER stress induces persistent CHOP expression but transient IRE1α activity, DR5 levels become a measure of the persistence of ER stress under the control of the opposing activities of PERK and IRE1α signaling, thus allowing time for adaptation before committing cells to apoptosis [56]. Therefore, cell fate decisions to ER stress and UPR activation are highly elaborate and context-dependent.

UPR in Cancer Metabolism

The process of tumor initiation and progression involves reprogramming of metabolic pathways. Compared with normal cells, cancer cells exhibit distinct metabolic profiles for glucose, glutamine, and lipids [125, 126]. Emerging evidences suggest that the ER can be perceived as a "nutrient-sensing" apparatus. The UPR coordinates the rates of protein synthesis with the supply of nutrients and energy. It also regulates cell metabolism to enable cancer cells to survive under nutrient-limiting conditions [100].

Lipid Metabolism

The synthesis of fatty acids is not only required for energy storage and the generation of signaling molecules, but also essential for membrane biosynthesis and cell proliferation [127]. Free fatty acids (FFAs) were shown to induce ER stress in pancreatic β-cells. Treatment of β-cells with palmitate, the most common long-chain saturated FFA in cells, caused ER disorganization and activation of all three UPR branches [128]. How palmitate induces ER stress was not clear. One suggested mechanism is that palmitate suppresses ER-to-Golgi trafficking, thus causing protein overloading in the ER lumen [129]. Under this circumstance, the introduction of BiP in β-cells protects against FFA-induced lipoapoptosis [130]. The mechanism underlying the ER stress-induced lipoapoptosis is unknown but may involve the induction of the proapoptotic CHOP transcription factor and the activation of JNK and Caspase-12 [131].

Several studies showed that ER stress activates sterol regulatory element binding protein 1 and 2 (SREBP1 and SREBP2), two master regulators of fatty acid and cholesterol synthesis, to promote de novo lipogenesis and meet the need for ER expansion [132–134]. A well-functioning UPR is also important for maintaining lipid homeostasis. After the challenge of tunicamycin, mice with genetic ablations of either UPR arms (*Ire1α*$^{-/-}$, *Atf6α* $^{-/-}$, eIF2α^{S51A}) displayed impaired resolution of ER stress and developed hepatic microvesicular steatosis, while the wild-type mice recovered to normal liver physiology after initial liver perturbations [135]. In those genetic mutant mice, the master regulators for lipogenesis (*Cebpa*, *Srebp1*, *Mlxipl*), fatty acid oxidation, and gluconeogenesis (*Ppara* and *Ppargc1a*) are significantly suppressed by persistent ER stress in the liver. The upregulation of the transcriptional repressor CHOP partially mediates this suppression of lipid homeostasis genes under prolonged ER stress in all the three genetic mutant mice [135]. How CHOP is induced independent of eIF2α phosphorylation remains to be elucidated. These findings reveal a direct link between ER homeostasis and the metabolic gene expression network.

Glutamine Metabolism

Glutamine is a crucial nutrient for cancer cells. It serves as a nitrogen and carbon source for the synthesis of macromolecules and, through the conversion to α-ketoglutarate, as an ATP source via the tricarboxylic acid cycle (TCA) and oxidative phosphorylation. Many cancer cells exhibit increased dependence on glutamine for proliferation, supply of TCA intermediates, neutralization of ROS, and synthesis of lipid [78, 136].

Recently, Ronai et al. revealed chemotherapy-induced UPR as a novel strategy to target glutamine-dependent cancer cells. Paclitaxel treatment induces the phosphorylation of IRE1α and eIF2α in MDA-MB-231 cells. This paclitaxel-triggered UPR induced the activity of RNF5, an ER-associated E3 ubiquitin ligase and a component of the ERAD. Activated RNF5 promoted the degradation of the glutamine transporters SLC1A5 and SLC38A2, reduced glutamine uptake and inhibited the mTOR signaling that led to the activation of autophagy and cell death [137]. A better understanding of the molecular connection between the UPR and glutamine metabolism is vital to the application of UPR-targeting therapy in treating glutamine-dependent cancers.

UPR in Cell–Cell Communication and the Tumor Microenvironment

All secreted and transmembrane proteins are synthesized and matured inside the ER lumen, thus the ER protein quality control mechanism plays important roles in cell–cell communication in the tumor microenvironment [8, 61]. Activation of the UPR in cancer cells actively promotes an angiogenic and proinflammatory milieu [138–141]. For example, XBP1 regulates a hypoxia-responsive transcriptional program

through its interaction with HIF1α [5]. Both the IRE1α-XBP1 and PERK/eIF2α branches have been shown to enhance the transcription of pro-angiogenic factors including *VEGFA*, *FGF2*, and *IL6*, and promote the adaptation of tumor cells to hypoxic stress [142–144]. Conversely, VEGF triggers the activation of all three UPR arms in endothelial cells through PLCγ-mediated crosstalk with the mTORC1 complex without apparent induction of protein overload in the ER. Activated ATF6 and PERK signaling are essential for the survival of endothelial cells and the induction of angiogenesis in vivo [145]. Collectively, the UPR functions in both tumor cells and endothelial cells to promote angiogenesis.

A recent study indicates that ER stress is transmissible from tumor cells to immune cells. Soluble factors that are secreted by thapsigargin-treated tumor cells activate the UPR in macrophages partially through TLR4. The transmitted ER stress then upregulates the expression of proinflammatory cytokines IL-6 and the p19 subunit of IL-23 to promote tumor growth [146]. It is not clear what soluble molecules mediate this transmissible ER stress. The physiological relevance of this acute ER stress-induced dialogue between tumor cells and stromal cells remains elusive and warrants further investigation. Recently, Clarke and colleagues discovered that silencing of *BiP* inhibited fatty acid oxidation in breast cancer and caused accumulation of polyunsaturated fatty acids, including linoleic acid. Inhibition of BiP or linoleic acid treatment suppressed CD47 expression and increased macrophage infiltration in vivo, suggesting a novel role of BiP in regulating innate immunity [147]. Interestingly, lipid peroxidation byproducts constitutively activate *XBP1* splicing in ovarian cancer-associated dendritic cells (DCs). Activated XBP1s induces a triglyceride biosynthetic program and reprograms lipid metabolism in DCs, which compromises the antigen presentation capacity of DCs and blocks the activation of cytotoxic $CD8^+$ T cells [9, 148, 149]. These studies establish the UPR as a critical regulator mediating paracrine and autocrine signalings in the tumor microenvironment. Targeting the UPR represents a two-pronged approach to simultaneously suppress tumor cell growth and stimulate an immune response against these cells.

Targeting UPR in Cancer

Both insufficient and excessive UPR activation can be detrimental to cancer cells that have already adapted and fine-tuned their UPR to stress. In principle, two therapeutic strategies could be used to target UPR in cancer: inhibition and hyperactivation (Table 7.2) [7, 118, 150–160].

Cancer cells rely on chaperone proteins for survival as demonstrated by the cytotoxic effects of genetic or pharmacological inhibition of molecular chaperones. AUY922, an HSP90 inhibitor, induces apoptosis and delays tumor growth in both mesenchymal pancreatic cancer mouse models and patient-derived xenograft models. Notably, the therapeutic efficacy could be improved by combining AUY922 with gemcitabine, a standard-of-care chemotherapy in pancreatic cancer [108]. In

Table 7.2 Summary of small molecules targeting key UPR components in in vivo tumor models

Agent	Target	Cancer types	Effects	Reference
MKC-3946	IRE1α endonuclease domain	Multiple myeloma	Reduces tumor volume, prolongs overall survival, and synergizes with bortezomib	[7]
STF083010		Multiple myeloma, colon cancer	Reduces myeloma volume, suppresses the growth of colon cancers that express wild-type p53	[118, 150]
B-I09		B cell chronic lymphocytic leukemia	Suppresses leukemic progression	[151]
Toyocamycin		Multiple myeloma	Reduces tumor volume and sensitizes to bortezomib treatment	[152]
GSK2606414	PERK	Pancreatic tumor	Attenuates tumor growth	[153]
GSK2656157		Pancreatic tumor, multiple myeloma, liver cancer	Slows down the growth of pancreatic tumor and multiple myeloma and inhibits tumor angiogenesis; slows down liver tumor growth	[154, 155]
ISRIB	eIF2B (activating)	Pancreatic tumor	Does not affect tumor growth when treated alone, but synergizes with gemcitabine	[156]
Salubrinal	eIF2α phosphatases	Breast cancer, cholangiocarcinoma, colon cancer	Reduces breast tumor volume; slows down cholangiocarcinoma growth and synergizes with rapamycin; promotes growth of colon-CSCs-derived tumor, but suppresses the growth of such tumors when in combination with oxaliplatin	[157–159]
HA15	BiP	Melanoma	Reduces tumor volume	[160]

IRE1a inositol-requiring enzyme 1a; *PERK* protein kinase RNA (PKR)-like ER kinase; *eIF2B* eukaryotic translation initiation factor 2B; *eIF2α* eukaryotic translation initiation factor 2 alpha; *BiP* binding immunoglobulin protein

melanoma, HA15 is a newly discovered compound that targets BiP and induces unresolved ER stress to kill cancer cells and sensitizes the cells to BRAF inhibitor treatment [160].

We recently discovered that XBP1 plays a pivotal role in TNBC. XBP1 is required and necessary for tumor initiation, progression, and relapse of TNBC [5]. This served as a proof-of-concept for the notion that the inhibition of XBP1 may be a novel targeted therapy for TNBC by interfering with a cellular stress response pathway. Transcription factors are considered one of the most difficult drug targets due to their nuclear localization and non-enzymatic character. However, the unique signaling cascade of XBP1, its unconventional cytosolic splicing, and activation by

IRE1α, offers an exceptional opportunity for the introduction of this transcription factor as a therapeutic target. Although small molecule inhibitors of RNases are unprecedented, against all odds, a class of salicylaldehyde IRE1α RNase inhibitors was identified and optimized into a series of potent and selective small molecule inhibitors with high bioavailability and low toxicity [161]. Compounds of this chemical class (for example, STF-083010, 4μ8C, B-I09, MKC3946) were further validated by other research groups [162]. The next step towards drug development is to adopt a pharmacological approach with one of these compounds. However, the dissimilarities between pharmacological inhibition and genetic targeting should be considered. A pharmacological inhibition of the IRE1α RNase results in the depletion of *XBP1s* and a buildup of *XBP1u* rather than a global depletion of both *XBP1* isoforms in an *XBP1* knockdown or knockout context. Differences due to effects from parallel RIDD inhibition and compensatory kinase activation can also be expected.

Similar approaches can be envisioned through targeting of the PERK arm [163]. ISRIB is recently discovered as a potent inhibitor of ISR signaling with low toxicity. ISRIB induces or stabilizes eIF2B dimerization, increasing its guanine nucleotide exchange factor (GEF) activity and desensitizing it to inhibition by phosphorylated eIF2α. Increased GEF activity of eIF2B potently reverses the effects of eIF2α phosphorylation and restores the translation capacity of cells [164, 165]. ISRIB can enhance cognitive memory in rodents [166]. However, its effect in tumor treatment is not well studied. Given that TNBCs are highly secretory and dependent on PERK signaling for survival in hypoxic and nutrient-deficient microenvironment, ISRIB treatment may represent a good strategy for targeting TNBC and other highly secretory tumors.

The Achilles' heel of traditional targeted cancer therapies is that they will eventually develop resistance due to the genetic instability and cellular heterogeneity of cancer cells. Although targeting the UPR pathway is still a targeted therapy, it puts under siege the whole defense mechanism of all the cancer cells that were selected through the same stress response mechanism. Additionally, while the application of a UPR inhibitor alone may result in a small or even negligible anticancer effect, the combination of this inhibitor with single agents or even a cocktail of chemotherapies, especially with compounds that cause ER stress, might result in a robust synergism. This might be even more rampant under in vivo conditions due to the systemic effects of intra-tumoral cell–cell communication.

Future Directions

An expanding body of evidence has shown that the UPR plays important roles in tumor initiation and progression. Manipulating the UPR might be therapeutically beneficial for TNBC treatment. However, several fundamental questions remain to be addressed in order to rationally target the UPR and improve disease outcomes. Since both excessive and insufficient activation of the UPR can be detrimental to

cancer cells, judicious caution should be excised in the design of UPR-targeting therapies to avoid undue adverse effects. The precise mechanisms of excessive ER stress-induced cell death are not yet clear. The properties of tumors that enable a beneficial effect from UPR-targeting therapy remain to be identified as well. More studies are also needed for the identification of potential biomarkers to predict and follow patient response to UPR-targeting therapies. Although a variety of compounds have been developed to target the UPR, the in vivo validation of these compounds in patient-relevant TNBC models is still lacking. Since a monotherapy targeting the UPR alone is unlikely to eradicate tumors, combination therapies should be developed to improve the therapeutic efficacy. We are still at the early stages of understanding the systemic effects of the UPR and their implications on cancer in general and on TNBC in particular. There are strong indications that targeting the UPR in TNBC has the potential for the intervention of this aggressive disease.

Acknowledgements This work was supported by the National Cancer Institute (NCI) (1R37CA228304-01 and 5P50CA186784-03 SPORE Career Development Award to X. C.), Cancer Prevention and Research Institutes of Texas (CPRIT) (RR150009 to X. C.), and Susan G. Komen for the Cure (CCR16380871 to X. C.). The authors declare no potential conflicts of interest. The authors apologize to those whose work could not be cited owing to space limitations.

References

1. Dejeans N, Barroso K, Fernandez-Zapico ME, Samali A, Chevet E. Novel roles of the unfolded protein response in the control of tumor development and aggressiveness. Semin Cancer Biol. 2015;33:67–73.
2. Tameire F, Verginadis II, Koumenis C. Cell intrinsic and extrinsic activators of the unfolded protein response in cancer: mechanisms and targets for therapy. Semin Cancer Biol. 2015;33:3–15.
3. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer. 2014;14(9):581–97.
4. Clarke HJ, Chambers JE, Liniker E, Marciniak SJ. Endoplasmic reticulum stress in malignancy. Cancer Cell. 2014;25(5):563–73.
5. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature. 2014;508(7494):103–7.
6. Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, et al. Epithelial-to-mesenchymal transition activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress. Cancer Discov. 2014;4(6):702–15.
7. Mimura N, Fulciniti M, Gorgun G, Tai YT, Cirstea D, Santo L, et al. Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. Blood. 2012;119(24):5772–81.
8. Cubillos-Ruiz JR, Bettigole SE, Glimcher LH. Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. Cell. 2017;168(4):692–706.
9. Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell. 2015;161(7):1527–38.

10. Condamine T, Kumar V, Ramachandran IR, Youn JI, Celis E, Finnberg N, et al. ER stress regulates myeloid-derived suppressor cell fate through TRAIL-R-mediated apoptosis. J Clin Invest. 2014;124(6):2626–39.
11. Hetz C, Chevet E, Harding HP. Targeting the unfolded protein response in disease. Nat Rev Drug Discov. 2013;12(9):703–19.
12. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol. 2000;2(6):326–32.
13. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334(6059):1081–6.
14. Schroder M, Kaufman RJ. The mammalian unfolded protein response. Annu Rev Biochem. 2005;74:739–89.
15. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007;8(7):519–29.
16. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell. 1999;10(11):3787–99.
17. Cox JS, Shamu CE, Walter P. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell. 1993;73(6):1197–206.
18. Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell. 1997;90(6):1031–9.
19. Mori K, Ma W, Gething MJ, Sambrook J. A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. Cell. 1993;74(4):743–56.
20. Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, et al. The unfolded protein response signals through high-order assembly of Ire1. Nature. 2009;457(7230):687–93.
21. Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F. Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. Cell. 2008;132(1):89–100.
22. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001;107(7):881–91.
23. Shen X, Ellis RE, Lee K, Liu CY, Yang K, Solomon A, et al. Complementary signaling pathways regulate the unfolded protein response and are required for C. elegans development. Cell. 2001;107(7):893–903.
24. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature. 2002;415(6867):92–6.
25. Cox JS, Walter P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell. 1996;87(3):391–404.
26. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol. 2003;23(21):7448–59.
27. Lu G, Ota A, Ren S, Franklin S, Rau CD, Ping P, et al. PPM1l encodes an inositol requiring-protein 1 (IRE1) specific phosphatase that regulates the functional outcome of the ER stress response. Mol Metab. 2013;2(4):405–16.
28. Ren S, Lu G, Ota A, Zhou ZH, Vondriska TM, Lane TF, et al. IRE1 phosphatase PP2Ce regulates adaptive ER stress response in the postpartum mammary gland. PLoS One. 2014;9(11):e111606.
29. Rubio C, Pincus D, Korennykh A, Schuck S, El-Samad H, Walter P. Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. J Cell Biol. 2011;193(1):171–84.
30. Prischi F, Nowak PR, Carrara M, Ali MM. Phosphoregulation of Ire1 RNase splicing activity. Nat Commun. 2014;5:3554.

31. Hollien J, Weissman JS. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. Science. 2006;313(5783):104–7.
32. Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci. 2014;39(5):245–54.
33. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 2000;287(5453):664–6.
34. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature. 1999;397(6716):271–4.
35. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, et al. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol Cell. 2001;7(6):1165–76.
36. Vattem KM, Wek RC. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc Natl Acad Sci U S A. 2004;101(31):11269–74.
37. Lu PD, Harding HP, Ron D. Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. J Cell Biol. 2004;167(1):27–33.
38. Jousse C, Oyadomari S, Novoa I, Lu P, Zhang Y, Harding HP, et al. Inhibition of a constitutive translation initiation factor 2alpha phosphatase, CReP, promotes survival of stressed cells. J Cell Biol. 2003;163(4):767–75.
39. Ma Y, Hendershot LM. Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. J Biol Chem. 2003;278(37):34864–73.
40. Lee YY, Cevallos RC, Jan E. An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation. J Biol Chem. 2009;284(11):6661–73.
41. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, et al. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev. 1998;12(7):982–95.
42. Chitnis NS, Pytel D, Bobrovnikova-Marjon E, Pant D, Zheng H, Maas NL, et al. miR-211 is a prosurvival microRNA that regulates chop expression in a PERK-dependent manner. Mol Cell. 2012;48(3):353–64.
43. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol. 2003;23(20):7198–209.
44. Zhang W, Hietakangas V, Wee S, Lim SC, Gunaratne J, Cohen SM. ER stress potentiates insulin resistance through PERK-mediated FOXO phosphorylation. Genes Dev. 2013;27(4):441–9.
45. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell. 2000;6(6):1355–64.
46. Guan D, Wang H, Li VE, Xu Y, Yang M, Shen Z. N-glycosylation of ATF6beta is essential for its proteolytic cleavage and transcriptional repressor function to ATF6alpha. J Cell Biochem. 2009;108(4):825–31.
47. Wu J, Rutkowski DT, Dubois M, Swathirajan J, Saunders T, Wang J, et al. ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell. 2007;13(3):351–64.
48. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. Dev Cell. 2007;13(3):365–76.
49. Yamamoto K, Yoshida H, Kokame K, Kaufman RJ, Mori K. Differential contributions of ATF6 and XBP1 to the activation of endoplasmic reticulum stress-responsive cis-acting elements ERSE, UPRE and ERSE-II. J Biochem. 2004;136(3):343–50.
50. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M, et al. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol Cell Biol. 2000;20(18):6755–67.

51. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell. 2006;124(3):587–99.
52. Saito A, Kanemoto S, Zhang Y, Asada R, Hino K, Imaizumi K. Chondrocyte proliferation regulated by secreted luminal domain of ER stress transducer BBF2H7/CREB3L2. Mol Cell. 2014;53(1):127–39.
53. Kondo S, Saito A, Hino S, Murakami T, Ogata M, Kanemoto S, et al. BBF2H7, a novel transmembrane bZIP transcription factor, is a new type of endoplasmic reticulum stress transducer. Mol Cell Biol. 2007;27(5):1716–29.
54. Kondo S, Murakami T, Tatsumi K, Ogata M, Kanemoto S, Otori K, et al. OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. Nat Cell Biol. 2005;7(2):186–94.
55. Brewer JW. Regulatory crosstalk within the mammalian unfolded protein response. Cell Mol Life Sci. 2014;71(6):1067–79.
56. Lu M, Lawrence DA, Marsters S, Acosta-Alvear D, Kimmig P, Mendez AS, et al. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis. Science. 2014;345(6192):98–101.
57. Walter F, Schmid J, Dussmann H, Concannon CG, Prehn JH. Imaging of single cell responses to ER stress indicates that the relative dynamics of IRE1/XBP1 and PERK/ATF4 signalling rather than a switch between signalling branches determine cell survival. Cell Death Differ. 2015;22(9):1502–16.
58. Gupta A, Hossain MM, Miller N, Kerin M, Callagy G, Gupta S. NCOA3 coactivator is a transcriptional target of XBP1 and regulates PERK-eIF2alpha-ATF4 signalling in breast cancer. Oncogene. 2016;35(45):5860–71.
59. Tsuru A, Imai Y, Saito M, Kohno K. Novel mechanism of enhancing IRE1alpha-XBP1 signalling via the PERK-ATF4 pathway. Sci Rep. 2016;6:24217.
60. Teske BF, Wek SA, Bunpo P, Cundiff JK, McClintick JN, Anthony TG, et al. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Mol Biol Cell. 2011;22(22):4390–405.
61. Bettigole SE, Glimcher LH. Endoplasmic reticulum stress in immunity. Annu Rev Immunol. 2015;33:107–38.
62. Hennighausen L, Robinson GW. Information networks in the mammary gland. Nat Rev Mol Cell Biol. 2005;6(9):715–25.
63. Zhu G, Wang M, Spike B, Gray PC, Shen J, Lee SH, et al. Differential requirement of GRP94 and GRP78 in mammary gland development. Sci Rep. 2014;4:5390.
64. Hasegawa D, Calvo V, Avivar-Valderas A, Lade A, Chou HI, Lee YA, et al. Epithelial Xbp1 is required for cellular proliferation and differentiation during mammary gland development. Mol Cell Biol. 2015;35(9):1543–56.
65. Davis KR, Giesy SL, Long Q, Krumm CS, Harvatine KJ, Boisclair YR. XBP1 regulates the biosynthetic capacity of the mammary gland during lactation by controlling epithelial expansion and endoplasmic reticulum formation. Endocrinology. 2016;157(1):417–28.
66. Bobrovnikova-Marjon E, Hatzivassiliou G, Grigoriadou C, Romero M, Cavener DR, Thompson CB, et al. PERK-dependent regulation of lipogenesis during mouse mammary gland development and adipocyte differentiation. Proc Natl Acad Sci U S A. 2008;105(42):16314–9.
67. Lin JH, Walter P, Yen TS. Endoplasmic reticulum stress in disease pathogenesis. Annu Rev Pathol. 2008;3:399–425.
68. Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, et al. Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell. 2005;122(1):33–43.
69. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747–52.
70. Cook KL, Shajahan AN, Warri A, Jin L, Hilakivi-Clarke LA, Clarke R. Glucose-regulated protein 78 controls cross-talk between apoptosis and autophagy to determine antiestrogen responsiveness. Cancer Res. 2012;72(13):3337–49.

71. Clarke R, Cook KL. Unfolding the role of stress response signaling in endocrine resistant breast cancers. Front Oncol. 2015;5:140.
72. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang HB, et al. NF-kappaB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol. 2015;35(2):379–90.
73. Rajapaksa G, Nikolos F, Bado I, Clarke R, Gustafsson JA, Thomas C. ERbeta decreases breast cancer cell survival by regulating the IRE1/XBP-1 pathway. Oncogene. 2015;34(31):4130–41.
74. Gomez BP, Riggins RB, Shajahan AN, Klimach U, Wang A, Crawford AC, et al. Human X-box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. FASEB J. 2007;21(14):4013–27.
75. Clarke R, Shajahan AN, Wang Y, Tyson JJ, Riggins RB, Weiner LM, et al. Endoplasmic reticulum stress, the unfolded protein response, and gene network modeling in antiestrogen resistant breast cancer. Horm Mol Biol Clin Investig. 2011;5(1):35–44.
76. Cook KL, Clarke PA, Parmar J, Hu R, Schwartz-Roberts JL, Abu-Asab M, et al. Knockdown of estrogen receptor-alpha induces autophagy and inhibits antiestrogen-mediated unfolded protein response activation, promoting ROS-induced breast cancer cell death. FASEB J. 2014;28(9):3891–905.
77. Cook KL, Clarke R. Estrogen receptor-alpha signaling and localization regulates autophagy and unfolded protein response activation in ER+ breast cancer. Receptors Clin Investig. 2014;1(6):e316.
78. Shajahan-Haq AN, Cook KL, Schwartz-Roberts JL, Eltayeb AE, Demas DM, Warri AM, et al. MYC regulates the unfolded protein response and glucose and glutamine uptake in endocrine resistant breast cancer. Mol Cancer. 2014;13:239.
79. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674–90.
80. Denkert C, Liedtke C, Tutt A, von Minckwitz G. Molecular alterations in triple-negative breast cancer—the road to new treatment strategies. Lancet. 2017;389:2430–42.
81. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;121(7):2750–67.
82. Perou CM. Molecular stratification of triple-negative breast cancers. Oncologist. 2011;16(Suppl 1):61–70.
83. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61–70.
84. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature. 2012;486(7403):405–9.
85. Wang J, Gao X, Qin P, He H, Cai ZG, Mu GY, et al. Expression of glucose regulated protein 78 in human triple negative breast cancer and its clinical implications. Int J Clin Exp Pathol. 2016;9(3):3134–42.
86. Zheng YZ, Cao ZG, Hu X, Shao ZM. The endoplasmic reticulum stress markers GRP78 and CHOP predict disease-free survival and responsiveness to chemotherapy in breast cancer. Breast Cancer Res Treat. 2014;145(2):349–58.
87. Guo L, Chi Y, Xue J, Ma L, Shao Z, Wu J. Phosphorylated eIF2alpha predicts disease-free survival in triple-negative breast cancer patients. Sci Rep. 2017;7:44674.
88. Davies MP, Barraclough DL, Stewart C, Joyce KA, Eccles RM, Barraclough R, et al. Expression and splicing of the unfolded protein response gene XBP-1 are significantly associated with clinical outcome of endocrine-treated breast cancer. Int J Cancer. 2008;123(1):85–8.
89. Andruska N, Zheng X, Yang X, Helferich WG, Shapiro DJ. Anticipatory estrogen activation of the unfolded protein response is linked to cell proliferation and poor survival in estrogen receptor alpha-positive breast cancer. Oncogene. 2015;34(29):3760–9.
90. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer. 2011;11(6):393–410.

91. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer. 2008;8(11):851–64.
92. Koritzinsky M, Levitin F, van den Beucken T, Rumantir RA, Harding NJ, Chu KC, et al. Two phases of disulfide bond formation have differing requirements for oxygen. J Cell Biol. 2013;203(4):615–27.
93. Koumenis C, Naczki C, Koritzinsky M, Rastani S, Diehl A, Sonenberg N, et al. Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2. Mol Cell Biol. 2002;22(21):7405–16.
94. Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, et al. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. Cancer Res. 2004;64(17):5943–7.
95. Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, et al. PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. Proc Natl Acad Sci U S A. 2013;110(12):4622–7.
96. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, et al. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. EMBO J. 2005;24(19):3470–81.
97. Koditz J, Nesper J, Wottawa M, Stiehl DP, Camenisch G, Franke C, et al. Oxygen-dependent ATF-4 stability is mediated by the PHD3 oxygen sensor. Blood. 2007;110(10):3610–7.
98. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell. 2003;11(3):619–33.
99. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress response. EMBO Rep. 2016;17(10):1374–95.
100. Kaufman RJ, Scheuner D, Schroder M, Shen X, Lee K, Liu CY, et al. The unfolded protein response in nutrient sensing and differentiation. Nat Rev Mol Cell Biol. 2002;3(6):411–21.
101. Dong J, Qiu H, Garcia-Barrio M, Anderson J, Hinnebusch AG. Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain. Mol Cell. 2000;6(2):269–79.
102. Hinnebusch AG. Translational regulation of GCN4 and the general amino acid control of yeast. Annu Rev Microbiol. 2005;59:407–50.
103. Moore CE, Omikorede O, Gomez E, Willars GB, Herbert TP. PERK activation at low glucose concentration is mediated by SERCA pump inhibition and confers preemptive cytoprotection to pancreatic beta-cells. Mol Endocrinol. 2011;25(2):315–26.
104. Lytton J, Westlin M, Hanley MR. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. J Biol Chem. 1991;266(26):17067–71.
105. Iurlaro R, Puschel F, Leon-Annicchiarico CL, O'Connor H, Martin SJ, Palou-Gramon D, et al. Glucose deprivation induces ATF4-mediated apoptosis through TRAIL death receptors. Mol Cell Biol. 2017;37(10):e00479–16.
106. Zeng L, Lu M, Mori K, Luo S, Lee AS, Zhu Y, et al. ATF6 modulates SREBP2-mediated lipogenesis. EMBO J. 2004;23(4):950–8.
107. Muaddi H, Majumder M, Peidis P, Papadakis AI, Holcik M, Scheuner D, et al. Phosphorylation of eIF2alpha at serine 51 is an important determinant of cell survival and adaptation to glucose deficiency. Mol Biol Cell. 2010;21(18):3220–31.
108. Genovese G, Carugo A, Tepper J, Robinson FS, Li L, Svelto M, et al. Synthetic vulnerabilities of mesenchymal subpopulations in pancreatic cancer. Nature. 2017;542(7641):362–6.
109. Babour A, Bicknell AA, Tourtellotte J, Niwa M. A surveillance pathway monitors the fitness of the endoplasmic reticulum to control its inheritance. Cell. 2010;142(2):256–69.
110. Koh CM, Sabo A, Guccione E. Targeting MYC in cancer therapy: RNA processing offers new opportunities. BioEssays. 2016;38(3):266–75.
111. Kress TR, Sabo A, Amati B. MYC: connecting selective transcriptional control to global RNA production. Nat Rev Cancer. 2015;15(10):593–607.

112. Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, et al. ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest. 2012;122(12):4621–34.
113. Horiuchi D, Kusdra L, Huskey NE, Chandriani S, Lenburg ME, Gonzalez-Angulo AM, et al. MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J Exp Med. 2012;209(4):679–96.
114. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer. 2011;11(11):761–74.
115. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov. 2014;13(2):140–56.
116. Denoyelle C, Abou-Rjaily G, Bezrookove V, Verhaegen M, Johnson TM, Fullen DR, et al. Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. Nat Cell Biol. 2006;8(10):1053–63.
117. Vazquez A, Bond EE, Levine AJ, Bond GL. The genetics of the p53 pathway, apoptosis and cancer therapy. Nat Rev Drug Discov. 2008;7(12):979–87.
118. Namba T, Chu K, Kodama R, Byun S, Yoon KW, Hiraki M, et al. Loss of p53 enhances the function of the endoplasmic reticulum through activation of the IRE1alpha/XBP1 pathway. Oncotarget. 2015;6(24):19990–20001.
119. Zhang F, Hamanaka RB, Bobrovnikova-Marjon E, Gordan JD, Dai MS, Lu H, et al. Ribosomal stress couples the unfolded protein response to p53-dependent cell cycle arrest. J Biol Chem. 2006;281(40):30036–45.
120. Brewer JW, Diehl JA. PERK mediates cell-cycle exit during the mammalian unfolded protein response. Proc Natl Acad Sci U S A. 2000;97(23):12625–30.
121. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest. 2005;115(10):2656–64.
122. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 2012;13(2):89–102.
123. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, et al. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP 1LC3B and ATG5. J Clin Invest. 2010;120(1):127–41.
124. Avivar-Valderas A, Salas E, Bobrovnikova-Marjon E, Diehl JA, Nagi C, Debnath J, et al. PERK integrates autophagy and oxidative stress responses to promote survival during extracellular matrix detachment. Mol Cell Biol. 2011;31(17):3616–29.
125. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2016;23(1):27–47.
126. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
127. Rohrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer. 2016;16(11):732–49.
128. Karaskov E, Scott C, Zhang L, Teodoro T, Ravazzola M, Volchuk A. Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic beta-cell apoptosis. Endocrinology. 2006;147(7):3398–407.
129. Preston AM, Gurisik E, Bartley C, Laybutt DR, Biden TJ. Reduced endoplasmic reticulum (ER)-to-Golgi protein trafficking contributes to ER stress in lipotoxic mouse beta cells by promoting protein overload. Diabetologia. 2009;52(11):2369–73.
130. Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV, et al. Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. Diabetologia. 2007;50(4):752–63.
131. Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL. Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology. 2004;145(11):5087–96.
132. Oh YS, Lee YJ, Kang Y, Han J, Lim OK, Jun HS. Exendin-4 inhibits glucolipotoxic ER stress in pancreatic beta cells via regulation of SREBP1c and C/EBPbeta transcription factors. J Endocrinol. 2013;216(3):343–52.

133. Colgan SM, Tang D, Werstuck GH, Austin RC. Endoplasmic reticulum stress causes the activation of sterol regulatory element binding protein-2. Int J Biochem Cell Biol. 2007;39(10):1843–51.
134. Lee JS, Mendez R, Heng HH, Yang ZQ, Zhang K. Pharmacological ER stress promotes hepatic lipogenesis and lipid droplet formation. Am J Transl Res. 2012;4(1):102–13.
135. Rutkowski DT, Wu J, Back SH, Callaghan MU, Ferris SP, Iqbal J, et al. UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. Dev Cell. 2008;15(6):829–40.
136. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16(10):619–34.
137. Jeon YJ, Khelifa S, Ratnikov B, Scott DA, Feng Y, Parisi F, et al. Regulation of glutamine carrier proteins by RNF5 determines breast cancer response to ER stress-inducing chemotherapies. Cancer Cell. 2015;27(3):354–69.
138. Binet F, Sapieha P. ER stress and angiogenesis. Cell Metab. 2015;22(4):560–75.
139. Mahadevan NR, Zanetti M. Tumor stress inside out: cell-extrinsic effects of the unfolded protein response in tumor cells modulate the immunological landscape of the tumor microenvironment. J Immunol. 2011;187(9):4403–9.
140. Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P. ER stress-induced inflammation: does it aid or impede disease progression? Trends Mol Med. 2012;18(10):589–98.
141. Maurel M, Samali A, Chevet E. Endoplasmic reticulum stress: at the crossroads of inflammation and metabolism in hepatocellular carcinoma development. Cancer Cell. 2014;26(3):301–3.
142. Auf G, Jabouille A, Guerit S, Pineau R, Delugin M, Bouchecareilh M, et al. Inositol-requiring enzyme 1alpha is a key regulator of angiogenesis and invasion in malignant glioma. Proc Natl Acad Sci U S A. 2010;107(35):15553–8.
143. Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, et al. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol Cell Biol. 2006;26(24):9517–32.
144. Wang Y, Alam GN, Ning Y, Visioli F, Dong Z, Nor JE, et al. The unfolded protein response induces the angiogenic switch in human tumor cells through the PERK/ATF4 pathway. Cancer Res. 2012;72(20):5396–406.
145. Karali E, Bellou S, Stellas D, Klinakis A, Murphy C, Fotsis T. VEGF signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. Mol Cell. 2014;54(4):559–72.
146. Mahadevan NR, Rodvold J, Sepulveda H, Rossi S, Drew AF, Zanetti M. Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. Proc Natl Acad Sci U S A. 2011;108(16):6561–6.
147. Cook KL, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, Warri A, et al. Endoplasmic reticulum stress protein GRP78 modulates lipid metabolism to control drug sensitivity and antitumor immunity in breast cancer. Cancer Res. 2016;76(19):5657–70.
148. Cubillos-Ruiz JR, Bettigole SE, Glimcher LH. Molecular pathways: immunosuppressive roles of IRE1alpha-XBP1 signaling in dendritic cells of the tumor microenvironment. Clin Cancer Res. 2016;22(9):2121–6.
149. Cubillos-Ruiz JR, Glimcher LH. Targeting abnormal ER stress responses in tumors: a new approach to cancer immunotherapy. Oncoimmunology. 2016;5(3):e1098802.
150. Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, et al. Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. Blood. 2011;117(4):1311–4.
151. Tang CH, Ranatunga S, Kriss CL, Cubitt CL, Tao J, Pinilla-Ibarz JA, et al. Inhibition of ER stress-associated IRE-1/XBP-1 pathway reduces leukemic cell survival. J Clin Invest. 2014;124(6):2585–98.

152. Ri M, Tashiro E, Oikawa D, Shinjo S, Tokuda M, Yokouchi Y, et al. Identification of Toyocamycin, an agent cytotoxic for multiple myeloma cells, as a potent inhibitor of ER stress-induced XBP1 mRNA splicing. Blood cancer journal. 2012;2(7):e79.
153. Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, et al. Discovery of 7-Methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J Med Chem. 2012;55(16):7193–207.
154. Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013;73(6):1993–2002.
155. Vandewynckel YP, Laukens D, Bogaerts E, Paridaens A, Van den Bussche A, Verhelst X, et al. Modulation of the unfolded protein response impedes tumor cell adaptation to proteotoxic stress: a PERK for hepatocellular carcinoma therapy. Hepatol Int. 2015;9(1):93–104.
156. Palam LR, Gore J, Craven KE, Wilson JL, Korc M. Integrated stress response is critical for gemcitabine resistance in pancreatic ductal adenocarcinoma. Cell Death Dis. 2015;6:e1913.
157. Hamamura K, Minami K, Tanjung N, Wan Q, Koizumi M, Matsuura N, et al. Attenuation of malignant phenotypes of breast cancer cells through eIF2alpha-mediated downregulation of Rac1 signaling. Int J Oncol. 2014;44(6):1980–8.
158. Zhao X, Zhang C, Zhou H, Xiao B, Cheng Y, Wang J, et al. Synergistic antitumor activity of the combination of salubrinal and rapamycin against human cholangiocarcinoma cells. Oncotarget. 2016;7(51):85492–501.
159. Wielenga MC, Colak S, Heijmans J, van Lidth de Jeude JF, Rodermond HM, Paton JC, et al. ER-stress-induced differentiation sensitizes colon cancer stem cells to chemotherapy. Cell Rep. 2015;13(3):490–4.
160. Cerezo M, Lehraiki A, Millet A, Rouaud F, Plaisant M, Jaune E, et al. Compounds triggering ER stress exert anti-melanoma effects and overcome BRAF inhibitor resistance. Cancer Cell. 2016;29(6):805–19.
161. Jiang D, Niwa M, Koong AC. Targeting the IRE1alpha-XBP1 branch of the unfolded protein response in human diseases. Semin Cancer Biol. 2015;33:48–56.
162. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, et al. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. Proc Natl Acad Sci U S A. 2012;109(15):E869–78.
163. Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I. Targeting the translation machinery in cancer. Nat Rev Drug Discov. 2015;14(4):261–78.
164. Sidrauski C, Tsai JC, Kampmann M, Hearn BR, Vedantham P, Jaishankar P, et al. Pharmacological dimerization and activation of the exchange factor eIF2B antagonizes the integrated stress response. elife. 2015;4:e07314.
165. Sidrauski C, McGeachy AM, Ingolia NT, Walter P. The small molecule ISRIB reverses the effects of eIF2alpha phosphorylation on translation and stress granule assembly. Elife. 2015;4.
166. Sidrauski C, Acosta-Alvear D, Khoutorsky A, Vedantham P, Hearn BR, Li H, et al. Pharmacological brake-release of mRNA translation enhances cognitive memory. Elife. 2013;2:e00498.

Chapter 8
The Unfolded Protein Response as an Integrator of Response to Endocrine Therapy in Estrogen Receptor Positive Breast Cancer

Robert Clarke

Abstract Tumors expressing either estrogen receptor-alpha (ER; ESR1) and/or the progesterone receptor (PR) represent the most prevalent breast cancer molecular subtype. Patients diagnosed with this form of breast cancer are generally treated with a drug that targets estrogen receptor action. While these drugs are highly effective in improving overall survival for many of these patients, a significant proportion will eventually experience a recurrence of their breast cancer. For most of these women, recurrence will arise after their first 5 years of endocrine therapy. Since there would be no tumor to recur if the treatment had successfully eradicated all diseases, a significant component of the biology of recurrent ER+ breast cancer is related to drug resistance, whether present initially within the cells (de novo resistance) or acquired by the cells in response to treatment (acquired resistance). The acquisition of endocrine resistance is explored here from the perspective of the unfolded protein response (UPR), which plays a central role as a key integrator of response to these treatments. Evidence for upregulation of prosurvival UPR signaling in acquired resistance is presented, as are some of the cellular effects of blocking ER action that lead to UPR induction. From a systems biology perspective, integrative UPR signaling can coordinate several modular functions within breast cancer cells, including apoptosis, autophagy, and proliferation, which contribute directly to the determination of cell fate outcomes in response to treatment.

Keywords Acquired resistance · Antiestrogen · Apoptosis · Autophagy · Aromatase inhibitor · BCL2 · BECN1 · De novo resistance · Endoplasmic reticulum stress · GRP78 · Metabolism · Molecular signaling · Systems biology

R. Clarke (✉)
Department of Oncology, Georgetown University Medical Center, Washington, DC, USA
e-mail: clarker@georgetown.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_8

Introduction

In women, breast cancer is the single most frequently diagnosed cancer and the second most prevalent cause of cancer mortality (lung cancer is the highest) [1]. Breast cancer cells can exhibit a luminal or basal cell phenotype, most likely originating from the acquisition of transforming mutations in a common luminal progenitor cell type [2]. Tumors that arise from within the luminal cells of the breast generally express either estrogen receptor-alpha (ER; ESR1) and/or the progesterone receptor (PR). These ER+ breast cancers comprise approximately 70% of all newly diagnosed breast cancers and represent the most prevalent breast cancer molecular subtype. There are two other general molecular subtypes, those expressing the oncogene HER2 (~20%; some also express ER and/or PR) and triple negative breast cancers (TNBC; ~15%) that express neither ER, PR, nor HER2. The systemic treatment selected for each patient reflects, to some degree, the stage of disease at diagnosis and the molecular subtype. For molecular subtypes, ER+ breast cancers are usually treated with some form of endocrine therapy. Patients with HER2+ tumors would generally receive a treatment directed at blocking HER2 action—drugs like the monoclonal antibody Trastuzumab (Herceptin®) and the small molecule tyrosine kinase inhibitor Lapatininb (Tykerb®). Where these tumors also express ER and/or PR, an endocrine therapy can be clinically beneficial. Currently, there is no targeted molecular therapy for TNBCs. Breast cancer patients with this molecular subtype generally receive cytotoxic chemotherapy. Patients of any molecular subtype who have a relatively poor prognosis at diagnosis are also likely to receive cytotoxic chemotherapy, as might patients whose ER+ or HER2+ breast cancer recurs during or after first-line therapy.

The role of the UPR in resistance to systemic cytotoxic chemotherapies and to local radiotherapy is discussed elsewhere in this book [3]. In this chapter, the focus is on endocrine therapies and the role of the UPR in affecting responsiveness. Endocrine therapies include ovariectomy, the first such approach [4] but now relatively uncommon, and drugs that target ER action. Currently, standard of care for postmenopausal women is achieved by administering a drug that acts by directly blocking the biosynthesis of 17β-estradiol (E2) and so preventing ligand-activation of the receptor; these drugs are aromatase inhibitors like Letrozole (Femara®) or Anastrozole (Arimidex®). The current generation of aromatase inhibitors is highly effective at blocking enzyme activity. It seems unlikely that drugs with markedly better activity will become available. While less widely used, a reduction in estrogen production is also achieved by using luteinizing hormone-releasing hormone (LHRH) agonists like Goserelin (Zoladex®). Because of significant ovarian toxicity, aromatase inhibitors are given to premenopausal women infrequently. Concurrent suppression of ovarian activity with an LHRH agonist is generally required when an aromatase inhibitor is used premenopause.

The widespread efficacy of Tamoxifen (TAM; Nolvadex®) in ER+ breast cancer is a direct reflection of its pharmacology. The cumulative intratumor concentrations of the antiestrogenic metabolites of TAM are estimated to be at least tenfold higher

than the concentration of E2 and estrogenic metabolites [5, 6]. While it may appear counterintuitive, the E2 concentrations in tumors in postmenopausal women are as high as those breast tumors arising in premenopausal women [5, 7]. Breast tumors in postmenopausal women have access to several sources of estrogens and their metabolic precursors (androgens). For example, breast tumors can acquire circulating estrogens produced in peripheral tissues [8] and androgens produced by the adrenal glands. The last step in the bioconversion of androgens to estrogens is accomplished by the p450 aromatase enzyme (CYP19A1), which can convert the androgens androstenedione to estrone and testosterone to E2. The adipose tissue that comprises much of the stroma in breast tumors is one of several tissues that can effectively perform these bioconversions [9, 10]. Aromatase inhibitors that block E2 production, and antiestrogens that block the ability of E2 to activate ER and/or target ER for degradation, are highly effective treatments for postmenopausal ER+ breast cancer [11].

The antiestrogen TAM remains the first-line endocrine therapy of choice for most premenopausal breast cancer patients with tumors in the ER+ subgroup. Antiestrogens are generally grouped into one of two classes by their mechanism of action. TAM is the oldest antiestrogen in clinical use [12] and exemplifies the class of selective estrogen receptor modulators (SERMs). These drugs can be partial agonists that activate or inhibit ER, often in a tissue-specific manner. For example, TAM is generally an antagonist in the breast and an agonist in the endometrium [5]. The agonist activity induced by TAM in the endometrium largely accounts for the increased risk of developing endometrial cancers associated with long-term TAM use [13]. Other SERMs do not necessarily increase the risk of endometrial cancer, e.g., Raloxifene (Evista®) [14]. The class of selective estrogen receptor degraders (SERDs) is exemplified by Fulvestrant (Faslodex®; ICI 182780). These drugs compete with estrogen for binding to ER and, when bound to ER, can inhibit receptor dimerization and target the receptor protein for degradation [15, 16]. Acting as receptor antagonists, SERDs are often referred to as "pure antiestrogens." Like aromatase inhibitors, SERDs are rarely given to premenopausal women and they require concurrent ovarian suppression when this is done.

TAM significantly reduces the risk of dying from breast cancer for those patients with ER+ tumors [17]. While aromatase inhibitors induce a longer disease-free survival than TAM, most studies show only minor differences in mortality rates at 10 years. For example, death without recurrence is not different for 5 years of TAM vs. 5 years of an aromatase inhibitor. Breast cancer mortality differs modestly at best, favoring an aromatase inhibitor by only 2.1% (10-year gain) [11]. Initial studies established Fulvestrant as being non-inferior to an aromatase inhibitor [18]. Most of these early comparisons were done using a dosing of 250 mg of the antiestrogen. Subsequently, 500 mg Fulvestrant was shown to be superior to 250 mg when given as a single agent [19]. Recent studies show better overall survival (not only disease-free survival) for Fulvestrant when compared with an aromatase inhibitor (for reviews, see [20, 21]). For example, Fulvestrant treatment confers a significant overall survival benefit relative to current aromatase inhibitors for patients diagnosed with metastatic ER+ disease [22]. It remains to be seen how long it will take

for SERDs to replace aromatase inhibitors as the preferred first-line endocrine therapy for all postmenopausal patients with ER+ breast cancer. Currently, Fulvestrant—often given with a CDK4,6 inhibitor like palboccilib (Ibrance®)—is used as the preferred second-line endocrine therapy following recurrence on an aromatase inhibitor [23].

The challenge with aromatase inhibitors and antiestrogens is that a significant number of women will eventually experience a recurrence of their ER+ breast cancer. Recurrent breast cancer is largely incurable, whether the recurrences are local or distant. The timing and risk of recurrence and death vary with stage at diagnosis. For example, patients diagnosed with T2 N4–9 ER+ disease have a 41% chance of experiencing a recurrence within 20 years of completing 5 years of an endocrine therapy. In contrast, patients diagnosed with T1N0 breast cancer have a 20-year recurrence risk of ~13% [24]. These patterns of late recurrence, often decades after completing 5 years of an endocrine therapy, are more common with ER+ disease than with TNBC [25].

Cytotoxic chemotherapy confers a survival benefit for breast cancer patients with a tumor in any of the molecular subtypes [26]. Pathological complete responses (pCR) to chemotherapy are often seen in breast cancer patients who exhibit a strong response, particularly for those with tumors in the TNBC or HER2+ molecular subtypes. A pCR in these patients often correlates with improved overall survival. In contrast, pCR is relatively uncommon in patients with ER+ disease and when detected is a poor predictor of overall survival [27]. It is not clear why pCR is rare and poorly predictive of overall survival in ER+ patients treated with an endocrine therapy, despite the clear overall survival benefit from these therapies [11]. ER+ breast tumors that respond well to an endocrine therapy are more likely to exhibit a dramatic drop in proliferation, as reflected in reduced Ki67 expression [28] or tumor shrinkage, than a pCR. One interpretation of these observations is that the induction of cell death by endocrine therapies occurs on a different time frame, and/or in fewer cells, particularly when compared with cytotoxic chemotherapy for rapidly proliferating tumors.

Molecular Features of Endocrine Resistance

Despite well over 100 years of experience with endocrine therapies, and almost 50 years since the advent of TAM, the molecular mechanisms driving resistance remain unclear. The clearest example of de novo resistance is lack of ER expression, yet most ER+ tumors that respond and then become resistant (acquired resistance) still express detectable levels of ER. Since responses to second- and third-line endocrine therapies are widely reported, ER is likely still to be a major driver of cell survival and proliferation in these tumors [29].

Since the ER is a nuclear transcription factor, the molecular signaling regulated by ER activation or inhibition has been the subject of extensive investigation for several decades. Many individual genes or pathways have been implicated (see for

reviews [29–31]). Nonetheless, disappointingly few of the many molecular targets identified in this notable body of work have led to new therapies or advanced as standard prognostic or predictive biomarkers to guide the management of ER+ disease. Our laboratories and those of our collaborators have added new candidates and/or confirmed the observations of others, adding to this long list. Genes our laboratories have implicated directly or indirectly in endocrine resistance include, e.g., AMPK [32], ATG5,9 [32, 33], BCL2 [34–38], BCL3 [35], BCLW [38], BCLxL [32], BECN1 (ATG6) [38], CASP7 [32, 39], CASP8 [40], GRP78 [32], GRP94 [39], IRF1, [34, 37, 41–45], KEAP1 [39], MYC [46, 47], NFκB [34–37, 40, 43, 48], NPM1 [34, 43, 49], NRF2 [39], STAT1 [37], mTOR [32], TSC1/2 [32], TOB1 [50], and XBP1 [34, 43, 46, 51]. Most of these genes either have been validated/extended by others or they represent our validation/extension of the work of others in the field.

Many of the genes noted above interact with features associated with UPR activation. For most of these genes, induction/inhibition by estrogen in sensitive cells, upregulation/downregulated in resistant cells (often constitutively), and/or a univariate association with clinical responses in patient-derived data sets, are often reported. Moreover, several genes in this listing are direct contributors to the resistance phenotype, either conferring resistance on sensitive cells and/or reversing the phenotype of resistant cells when appropriately manipulated by RNAi or cDNA transfection and/or by small molecules. New candidate molecules or small signaling features that meet these various research criteria are continually being reported; not all of these entirely fail to live up to expectations. For example, among those reported over the years, targeting the altered function of mTOR by Everolimus (Afinitor®) has been clinically useful, with evidence of its inhibition having clinical benefit for some patients with ER+ disease although not substantially extending overall survival [52, 53]. When considered together, these observations imply that the endocrine resistance phenotype may have multiple molecular contributors and exhibit a remarkable degree of plasticity.

Other groups have looked for a contribution to endocrine resistance from the genes that are more frequently mutated in ER+ breast cancer, mostly PIK3CA, AKT1, and PTEN. Several drugs that target these mutations have been tested in clinical trials but provided somewhat limited (if any) overall survival benefit for patients with recurrent, ER+ disease. ESR1 mutations are logical candidates for a mutational explanation of endocrine resistance. However, ESR1 mutations are rare in primary ER+ tumors and in tumors that recur following antiestrogen therapy. In contrast, ~30% of patients recurring following a first-line aromatase inhibitor have tumors with detectable ESR1 mutations [29]. While the presence of ESR1 mutations likely explains resistance to the aromatase inhibitor, it would be uncommon to switch to treatment with another aromatase inhibitor. Moreover, patients treated with an aromatase inhibitor are rarely selected for treatment on the basis of detectable ESR1 mutations yet, upon recurrence, receive significant clinical benefit from a second-line antiestrogen (TAM or Fulvestrant), often given with a CDK4,6 inhibitor like palbociclib (Ibrance®) [54–56]. Thus, the predictive and/or prognostic value of ESR1 mutations is unclear and requires additional study.

While several of the driver-mutated genes noted above are relatively common in ER+ breast tumors, none has yet shown an overall survival benefit from a targeted intervention comparable to that experienced by targeting HER2 in HER2+ breast tumors. Currently, no driver mutation(s) has been found with the prevalence and clinical activity needed solely to explain endocrine resistance. Taken together, these observations suggest that endocrine resistance may be more often acquired by cellular adaptation than by a new gene mutation. This conclusion does not exclude the possibility that mutations in some genes alter the probability that resistance will arise. For example, a gene mutation may make it easier for a cell to adapt by activation (or repression) of other signaling features that affect the cell fate decision or execution process(s).

Despite progress in identifying new molecular targets, some of which have shown clinical utility, recurrent ER+ breast cancer remains largely incurable. When combined with an endocrine therapy, these new treatments for metastatic disease generally offer only a few months or so of increased survival, and often with dose-limiting toxicities. For example, adding an mTOR inhibitor to an endocrine therapy has clear progression-free survival benefit (but not overall survival) but with added toxicity that may require dose modification in some patients [52, 57]. When considered in the light of the nature of the basic and translational experimental evidence originally supporting the evaluation of these targets, this general observation implies that a reductionist view of resistance, where the phenotype can be explained by a single (or few) acquired mutation or an altered canonical signal transduction pathway, may be one limiting factor. Tumor heterogeneity likely also contributes to the modest impact of these newer combination regimens on overall survival. This chapter takes a more systems-based perspective on endocrine resistance, with a focus on key aspects of an activated UPR.

The Unfolded Protein Response

The UPR is an ancient cellular response that can drive development [58] or response to a diverse range of stressors [3, 59, 60]. In its canonical form, maps of the UPR network depict three signaling arms controlled, respectively, by IRE1α (inositol-requiring enzyme-1 alpha), ATF6 (activating transcription factor-6), and PERK (protein kinase R(PKR)-like endoplasmic reticulum kinase) [61]. Each of these three sensors are normally retained as inactive, endoplasmic reticulum (EnR) membrane resident proteins because of their binding to GRP78 (glucose regulated protein-78), a molecular chaperone of unfolded or misfolded proteins [62]. When a cell is unable to match its ability to fold nascent proteins into their appropriate conformations, as may occur when there is insufficient energy available, the accumulation of unfolded proteins in the EnR causes a swelling of the structure and induction of EnR stress. The UPR is activated in response to this proteotoxic stress and provides the cell with an integrated network of signaling that coordinates and regulates cell functions to help restore homeostasis. A similar process can be initiated in

mitochondria in response to the accumulation of misfolded or damaged proteins [63]. In response to heat shock, activation of the UPR is very rapid and appears to be prewired [64]. This rapid activation, which is too fast for new RNA or protein synthesis, is called an anticipatory UPR response [65]. Breast cancer cells also exhibit this anticipatory response with activation of ER [66].

UPR activation within the EnR is generally achieved by the release of the three UPR sensors from GRP78, which appears to bind preferentially the unfolded, misfolded, and/or damaged proteins that produced the EnR stress [67]. The three signaling arms act in an apparently coordinated manner to reduce the rates of transcription and translation, increase the production of chaperones to remove unfolded proteins, attempt to acquire sufficient energy to fold those remaining proteins and to ensure cell survival and function, while also restraining signaling that could lead to induction of an irreversible programmed cell death activity such as apoptosis [60]. Depending on the nature of the stress or the factors inducing the UPR, each arm may not be fully engaged, or may engage on different time frames. For example, each arm contributes differently to driving breast cancer cell growth compared with inducing or maintaining the cell's stemness [68].

Following the canonical UPR model, activation of the PERK arm is the primary regulator of global rates of transcription and translation. Upon release from GRP78, signaling proceeds from an activated PERK, produced by its oligomerization and autophosphorylation, to phosphorylation of eIF2α that then regulates initiation of mRNA translation. Thus, the primary functional consequence of this action of activated PERK signaling is a reduced rate of protein translation. Rapid activation of eIF2α is likely most important for blocking translation of existing mRNA transcripts, whereas prolonged PERK activity could regulate translation of newly synthesized transcripts. In proliferating cells, inhibition of translation can also cause the cells to arrest in G1 [69]. This arrest would limit the increased load for protein synthesis required if the cell was to exit G1 and commit to a full turn of the cell cycle.

While PERK reduces the protein load in the EnR from new proteins for folding, the EnR must still remove remaining unfolded, misfolded, or damaged proteins. Many of these activities are coordinated through the IRE1α and ATF6 signaling arms. Upon release from GRP78, the ATF6 protein (90 kDa) translocates to the Golgi and is cleaved by site-1 and site-2 proteases to an active transcription factor protein (ATF6; 50 kDa) that binds to the 5′-CCAC[GA]-3′ component of the EnR stress response element-I sequence (ERSE; 5′-CCAAT-N9-CCAC[GA]-3′) and of the ERSE II sequence (5′-ATTGG-N-CCACG-3′) [70, 71]. Co-binding of NF-Y at the ERSE is required for transcription to be initiated [72]. Activation of IRE1α leads to the unconventional splicing of the native XBP1 unsliced (XBP1U) transcript that removes a 26 bp intron and creating an active XBP1 transcription factor (XBP1-spliced; XBP1-S). XBP1-S binds to the ESRE [73], a consensus cAMP responsive element (CRE)-like sequence (5′-GATGACGTG[TG]N(3)[AT]T-3′) [74], to a 12-*O*-tetradecanoylphorbol 13-acetate)-response element (5′-TGAGTCAG-3′) [75], and within the long terminal repeat (LTR) of T-cell leukemia virus type 1 (the Tax-responsive element) [76, 77] that also contains a CRE (5′-CAGGCGTTGACGACAACCCCT-3′) [78, 79]. Together, activation of ATF6

(50 kDa) and XBP1 (XBP1-S) regulates the expression of many of the chaperones needed to execute the protein elimination activities of an active UPR.

Degradation of targeted proteins occurs through an EnR-associated protein degradation (ERAD) process that leads to protein ubiquitination and degradation through the ubiquitin proteasome [80]. Chaperone-marked proteins may also be degraded through components of a chaperone-mediated autophagy or macroautophagy [67]. The products of protein degradation can be fed back into intermediate metabolism, providing an energy efficient means to assist the cell in recovering metabolic homeostasis. For example, recycling of amino acids released by degradation would reduce the need for their biosynthesis, whereas select other amino acid molecules could enter the TCA cycle to generate additional ATP.

Regulation of the UPR in ER+ Breast Cancer

Previously, we proposed a mathematical modeling framework to capture how breast cancer cells respond to activation or inhibition of ER. This model, based on prior work from this and many other laboratories, invokes the coordinated regulation of a series of integrated modular functions including the UPR [81]. In principle, there are two forms of regulation of specific nodes in the UPR network. The first form of regulation is that captured in the canonical pathways—the regulation of individual UPR components in response to EnR (or mitochondrial) stress. A simple example of this form is the activation of IRE1α, ATF6, and/or PERK upon their release from GRP78. The second form of regulation is that driven by the ability of the ER to regulate directly genes within the UPR. An example of this regulation is the estrogenic response of XBP1 by ER binding to estrogen responsive elements within the XBP1 gene promoter [82]. Moreover, XBP1 can act as an ER coregulator and improve the efficacy of gene transcription when driven by ER as discussed elsewhere in this book [82].

Both forms of UPR regulation may be affected by the cellular context experienced by a breast cancer cell. For example, signaling that drives endocrine resistance is likely to be affected by cell-cell communication through juxtacrine interactions (such as gap junctions) and paracrine interactions that include the exchange of extracellular vesicles and/or secreted proteins [83, 84]. The breast tumor microenvironment is heterogeneous, dynamic [2, 85, 86], and therefore complex. Multiple cell types are present in ratios that likely differ over time and in response to treatments [87]. Thus, the changing concentrations of hormones, growth factors, chemokines, cytokines, and other proteins secreted into the microenvironment, and that can affect UPR signaling in the cancer cells, creates a dynamic and cell context-specific cancer cell-intrinsic system, where each cancer cell exists within a dynamic neoplastic ecosystem. For most antineoplastic drugs, it is the cancer cell intrinsic activities that are targeted and that execute an individual cell's fate.

Studying such a complex and dynamic system, even using relatively simple in vitro systems, is challenging [88]. As described earlier in this book, computa-

tional and mathematical modeling provides the opportunity to take a more systems-based approach [67]. The first framework for such studies includes several modular functions; the UPR is one of these modules [81]. Subsequently, we have begun to model small [45] and larger features of the endocrine responsive breast cancer cell system [89, 90]. For example, we built a phenomenological mathematical model of a small feature of the effects of Fulvestrant that comprised ER, IRF1, ATG7, and with proliferation as the output prediction [45]. The goal was to understand the role of IRF1, a putative tumor suppressor, in mediating the effects of Fulvestrant [42, 44, 92]. The model predicted that further increases in IRF1 knockdown (but not for ATG7 knockdown) would affect cell proliferation. We tested the model predictions using higher concentrations of RNAi targeting IRF1 and ATG7. The modeling and experimental outcomes taught us that ATG7 knockdown activates both an IRF1-dependent and an IRF1-independent mechanism to affect cell death [45].

Many of the genes noted above, that others and we have implicated as single gene drivers of the resistance phenotype, interact to create features within the UPR network. For example, UPR activation by GRP78 leads to increased splicing of XBP1, NFκB activation [48], and the regulation of degenerate signaling through the BCL2 family (e.g., BCL2, BCL3, BCLW) to coordinately suppress apoptosis and activate prosurvival autophagy (through BECN1, mTOR, AMPK) [32, 35, 37, 38, 92].

Figure 8.1 shows the main interacting network modules, i.e., apoptosis, autophagy, proliferation, UPR, cell metabolism, that comprise our current mathematical modeling framework. For endocrine responsiveness, signal flow is affected by endocrine therapies modifying cell metabolism, e.g., MYC regulation of glucose and glutamine to induce an energy deficit [47], G_0/G_1 arrest, and endoplasmic reticulum stress activation of GRP78 to induce the UPR [32, 39] (activation of PERK, IRE1, ATF6). UPR induction likely reflects insufficient energy to support protein folding in the endoplasmic reticulum [60]. Hence, some endocrine therapies could also be considered to be antimetabolites. The classical antimetabolites like methotrexate and 5-fluorouracil are enzyme inhibitors that block the production of cellular metabolites. Aromatase inhibitor blockade of estrogen biosynthesis leads to a loss of ER activation that results in reduced cellular metabolism [29].

Prodeath UPR outputs including PERK → CHOP → caspase activation and apoptosis [32, 37, 40] are balanced against prosurvival UPR outputs, such as those received from the GRP78 → IRE1α arm following XBP1 splicing [34, 46, 48, 93] that induces NFκB and anti-apoptotic BCL2 family members [34, 36, 48]. Several prosurvival BCL2 family members sequester BECN1 (repress prosurvival autophagy) [38, 94, 95] and may no longer be free to protect mitochondria (activate apoptosis). When there are sufficient prosurvival BCL2 activities, mitochondria are protected and prosurvival autophagy is driven independent of BECN1 [96] (if BECN1 is sequestered), for example, by GRP78 → AMPK/mTOR [32], and/or some BECN1 remains active. Degradation products from autophagy can then feed intermediate metabolism and reduce the energy expense of attempting to recover metabolic homeostasis in the face of the energy deficit induced by an endocrine therapy. Overall, the sum of prodeath and prosurvival signals determines cell fate (survival vs. death; proliferation vs. arrest). Changing this balance by blocking

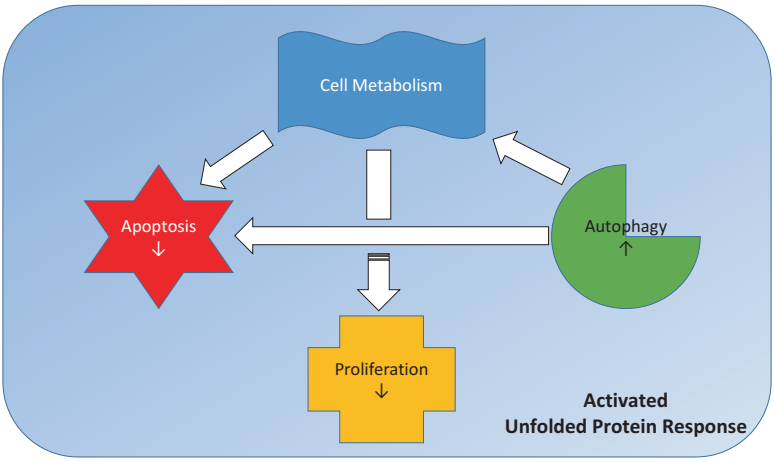

Fig. 8.1 Simple representation of the five cell function modules that sense and execute the coordinated response to an endocrine therapy in ER+ breast cancer cells. The unfolded protein response is a central module that can coordinate actions performed by each of the other four modules (apoptosis, autophagy, cell metabolism, proliferation). The endocrine therapy reduces intracellular energy reserves such that protein folding cannot be completed and the UPR is triggered. The UPR manages the balance between proliferation, apoptosis, and prosurvival autophagy. Hence, cells arrest in G0/G1 and the energy need to synthesize proteins to make a full copy of the cell is eliminated. Apoptosis is prevented while autophagy attempts to recover metabolic homeostasis through recycling damaged, misfolded, and unfolded proteins and excess/unnecessary organelles. Concurrently, the UPR also reduces the rates of transcription and translation to lower the overall energy demand for protein folding, while increasing the production of new chaperone proteins to manage the degradation of existing damaged, misfolded, and unfolded proteins. If homeostasis is reacquired, the breast cancer cell will survive and may eventually acquire sufficient energy to exit G0/G1 and reinitiate proliferation. Any long-term modifications to cellular signaling may also be epigenetically reprogrammed to ensure the survival and proliferation of parent and daughter cells. If homeostasis is not regained, the prolonged loss of organelles and proteins from sustained UPR and autophagy activation renders cell survival untenable. The block on apoptosis is released and the cell enters a programmed cell death fate, where there is sufficient energy. If there is insufficient energy to execute a programmed cell death, the cell may die from a more necrotic form of death

autophagy with the inhibitor chloroquine partially reverses antiestrogen resistance in vitro and in vivo and increases cell death in response to TAM or Fulvestrant [33].

In the context of endocrine resistance, those cells that can use this integrated, UPR-focused system to adapt to the stress of the treatment will survive. While many cells will be surviving but growth arrested [28], at least initially, the ability to epigenetically reprogram an upregulated stress response network that includes the UPR could allow their progeny, once they are able to resume cell cycling, to already exhibit an endocrine-resistant phenotype. Evidence of an epigenetic component of resistance has been obtained from both cell line and animal models of endocrine resistance [97].

The model in Fig. 8.1 is adaptive and does not require mutations to explain endocrine resistance. Nonetheless, mutations in the PIK3CA (34.5%), PTEN (2.3%),

and AKT1 (1.4%) genes occur in ER+ breast cancers [98], with ESR1 mutations arising in response to prolonged treatment with an aromatase inhibitor. For ESR1 mutations, it is not immediately clear why these are not widespread in primary tumors or with acquired resistance to antiestrogens. One possibility is that, since ER can become constitutively activated by growth factor signaling [99, 100], this event may be more prevalent, and arise earlier, than mutational events in ESR1. The most common ESR1 mutations appear to be insufficient to drive full resistance to an antiestrogen [29]. This observation may reflect the domains of the ER protein affected by these mutations, which could alter antiestrogen responsiveness [101]. However, the changes produced by these mutations may be insufficient to overcome the structural and functional changes in the ER protein conferred when an antiestrogen is still able to bind. For SERDs, the mutations may not substantially affect the ability of these drugs to target the ER protein for degradation.

Mutations in PIK3CA, PTEN, and AKT also appear inadequate to explain endocrine resistance fully [98]. The explanations here are different from those for ESR1 mutations. Feedback activation is known for inhibition of PI3K-AKT signaling [102], dampening the ability of the signaling to drive some downstream activities in the network. Moreover, the signaling affected here represents only one small component of the overall UPR network or relates primarily to only one of the replace features therein (Fig. 8.1). Redundancy could also make activation of this feature of network topology less important, such that other modules adapt to the signal from the mutations to limit the impact of the signaling affected by the mutant proteins. Thus, UPR network redundancy and signaling degeneracy within network modules may explain why mutations are relatively rare in ER+ breast cancers. While mutations are not necessary for resistance, in some cells specific mutations could increase the likelihood that altered signaling in one area of the network increases the probability that changes elsewhere will tip the balance in favor of cell survival over cell death. In contrast, epigenetic events may be critical for maintaining an adapted resistant phenotype once this has been acquired [97]. This observation is consistent with the concurrent changes in transcriptional reprogramming and chromatin remodeling that occurs with stress [64].

When placed in the context of normal mammary gland biology, activation of the UPR in response to the proteotoxic stress induced by an endocrine therapy is entirely logical. During lactation, the breast synthesizes, folds, and secretes large amounts of proteins during the production of milk. Balancing the prodeath and prosurvival signaling in favor of cell survival is likely pre-programmed into the mammary tissues of all mammals. The coordination of cell survival signaling by the UPR (such as activation of BCL2) and of the means to provide the energy for cell survival (such as induction of macroautophagy) helps to ensure that the excessive energy demands of protein production and folding do not trigger inappropriate cell death during lactation [103]. Thus, breast cancer cells stressed by an endocrine therapy may also be pre-programmed to use the same UPR-coordinated activities to survive and proliferate [104].

Conclusions and Future Prospects

The UPR plays a significant role in coordinating the response of ER+ breast cancer cells to the stresses of an endocrine therapy. The overall response system is likely more complex than is described in this chapter. Much remains to be understood regarding the balance of regulatory and integrative signaling that can be concurrently affected by ER-mediated signaling (cell context specific) and through the effects of the stress that can activate the UPR through its canonical signaling activities. Nonetheless, several of the genes listed in this chapter are likely to be good candidates for targeted interventions. For example, others have noted the potential of targeting GRP78 in cancer [105]. Identifying the most effective targets, and/or how drugs may be combined to shut down UPR network function in cancer cells safely and effectively, may prove difficult and require a greater understanding of the dynamical nature of the UPR network and its inherent redundancies and degeneracies. Cell context differences are likely critical if combinatorial drug regimens are to avoid significant host toxicity due to concurrent UPR inhibition in normal cells. Multiscale modeling may offer one approach to address these complexities; more complicated mathematical models of the UPR network seem essential in this regard.

One area of focus in the context of resistance will be to improve our understanding of how the UPR controls the various redundant cell death functions available. This chapter has discussed primarily apoptosis and autophagy but other related mechanisms exist including necroptosis, ferroptosis, and pyroptosis. Signaling to control and execute these cell death pathways is often highly integrated, with network pathways sharing common nodes but with different edges.

The role of the UPR in controlling the execution of cell fate decisions in cancer and normal cells clearly requires further study. The UPR may be a relatively ancient stress response [106] but only by applying the most modern and powerful tools available are we likely to uncover all of its secrets.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68:7–30.
2. Skibinski A, Kuperwasser C. The origin of breast tumor heterogeneity. Oncogene. 2015;34:5309–16.
3. Singleton DC, Harris AL. ATF4, hypoxia and treatment resistance in cancer. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
4. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions from a new method of treatment, with illustrative cases. Lancet. 1896;2:104–7.
5. Clarke R, Leonessa F, Welch JN, Skaar TC. Cellular and molecular pharmacology of antiestrogen action and resistance. Pharmacol Rev. 2001;53:25–71.
6. Nicholson RI, Hutcheson IR, Harper ME, Knowlden JM, Barrow D, McClelland RA, Jones HE, Wakeling AE, Gee JM. Modulation of epidermal growth factor receptor in endocrine-resistant, oestrogen receptor-positive breast cancer. Endocr Relat Cancer. 2001;8:175–82.
7. Clarke R, Liu MC, Bouker KB, Gu Z, Lee RY, Zhu Y, Skaar TC, Gomez B, O'Brien K, Wang Y, Hilakivi-Clarke LA. Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. Oncogene. 2003;22:7316–39.

8. Masamura S, Santner SJ, Gimotty P, Georger J, Santen RJ. Mechanism for maintenance of high breast tumor estradiol concentrations in the absence of ovarian function: role of very high affinity tissue uptake. Breast Cancer Res Treat. 1997;42:215–26.
9. Tilson-Mallett N, Santner SJ, Feil PD, Santen RJ. Biological significance of aromatase activity in human breast tumors. J Clin Endocrinol Metab. 1983;57:1125–8.
10. Santen RJ, Santner SJ, Pauley RJ, Tait L, Kaseta J, Demers LM, Hamilton C, Yue W, Wang JP. Estrogen production via the aromatase enzyme in breast carcinoma: which cell type is responsible? J Steroid Biochem Mol Biol. 1997;61:267–71.
11. Early Breast Cancer Trialists' Collaborative Group. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet. 2015;386:1341–52.
12. Cole MP, Jones CTA, Todd IDH. A new antioestrogenic agent in late breast cancer. An early clinical appraisal of ICI 46474. Br J Cancer. 1971;25:270–5.
13. Hu R, Hilakivi-Clarke L, Clarke R. Molecular mechanisms of tamoxifen-associated endometrial cancer. Oncol Lett. 2015;9:1495–501.
14. DeMichele A, Troxel AB, Berlin JA, Weber AL, Bunin GR, Turzo E, Schinnar R, Burgh D, Berlin M, Rubin SC, Rebbeck TR, Strom BL. Impact of raloxifene or tamoxifen use on endometrial cancer risk: a population-based case-control study. J Clin Oncol. 2008;26:4151–9.
15. Dauvois S, Danielian PS, White R, Parker MG. Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. Proc Natl Acad Sci U S A. 1992;89:4037–41.
16. Fawell SE, White R, Hoare S, Sydenham M, Page M, Parker MG. Inhibition of estrogen receptor-DNA binding by the "pure" antiestrogen ICI 164,384 appears to be mediated by impaired receptor dimerization. Proc Natl Acad Sci U S A. 1990;87:6883–7.
17. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet. 1998;351:1451–67.
18. Howell A, Robertson JF, Quaresma AJ, Aschermannova A, Mauriac L, Kleeberg UR, Vergote I, Erikstein B, Webster A, Morris C. Fulvestrant, formerly ICI 182,780, is as effective as anastrozole in postmenopausal women with advanced breast cancer progressing after prior endocrine treatment. J Clin Oncol. 2002;20:3396–403.
19. Di LA, Jerusalem G, Petruzelka L, Torres R, Bondarenko IN, Khasanov R, Verhoeven D, Pedrini JL, Smirnova I, Lichinitser MR, Pendergrass K, Garnett S, Lindemann JP, Sapunar F, Martin M. Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. J Clin Oncol. 2010;28:4594–600.
20. Nathan MR, Schmid P. A review of Fulvestrant in breast cancer. Oncol Ther. 2017;5:17–29.
21. Reinert T, Barrios CH. Overall survival and progression-free survival with endocrine therapy for hormone receptor-positive, HER2-negative advanced breast cancer: review. Ther Adv Med Oncol. 2017;9:693–709.
22. Robertson JF, Bondarenko IM, Trishkina E, Dvorkin M, Panasci L, Manikhas A, Shparyk Y, Cardona-Huerta S, Cheung KL, Philco-Salas MJ, Ruiz-Borrego M, Shao Z, Noguchi S, Rowbottom J, Stuart M, Grinsted LM, Fazal M, Ellis MJ. Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. Lancet. 2016;388:2997–3005.
23. Rugo HS, Rumble RB, Macrae E, Barton DL, Connolly HK, Dickler MN, Fallowfield L, Fowble B, Ingle JN, Jahanzeb M, Johnston SR, Korde LA, Khatcheressian JL, Mehta RS, Muss HB, Burstein HJ. Endocrine therapy for hormone receptor-positive metastatic breast bancer: American Society of Clinical Oncology guideline. J Clin Oncol. 2016;34:3069–103.
24. Pan H, Gray R, Braybrooke J, Davies C, Taylor C, McGale P, Peto R, Pritchard KI, Bergh J, Dowsett M, Hayes DF. 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N Engl J Med. 2017;377:1836–46.
25. Demicheli R, Ardoino I, Boracchi P, Coradini D, Agresti R, Ferraris C, Gennaro M, Hrushesky WJ, Biganzoli E. Recurrence and mortality according to estrogen receptor status for breast cancer patients undergoing conservative surgery. Ipsilateral breast tumour recurrence dynamics provides clues for tumour biology within the residual breast. BMC Cancer. 2010;10:656.

26. Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of randomised trials. Lancet. 1998;352:930–42.
27. Pennisi A, Kieber-Emmons T, Makhoul I, Hutchins L. Relevance of pathological complete response after neoadjuvant therapy for breast cancer. Breast Cancer. 2016;10:103–6.
28. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. J Clin Oncol. 2005;23:7212–20.
29. Clarke R, Tyson JJ, Dixon JM. Endocrine resistance in breast cancer—an overview and update. Mol Cell Endocrinol. 2015;418:220–34.
30. Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. Nat Rev Cancer. 2009;9:631–43.
31. Bogush TA, Polezhaev BB, Mamichev IA, Bogush EA, Polotsky BE, Tjulandin SA, Ryabov AB. Tamoxifen never ceases to amaze: new findings on non-estrogen receptor molecular targets and mediated effects. Cancer Investig. 2018;36:211–20.
32. Cook KL, Shajahan AN, Jin L, Warri A, Hilakivi-Clarke LA, Clarke R. Glucose-regulated protein 78 controls cross-talk between apoptotsis and autophagy to determine antiestrogen responsiveness. Cancer Res. 2012;72:3337–49.
33. Cook KL, Wärri A, Soto-Pantoja DR, Clarke PAG, Cruz MI, Zwart A, Clarke R. Chloroquine inhibits autophagy to potentiate antiestrogen responsiveness in ER+ breast cancer. Clin Cancer Res. 2014;20:3222–32.
34. Gu Z, Lee RY, Skaar TC, Bouker KB, Welch JN, Lu J, Liu A, Zhu Y, Davis N, Leonessa F, Brunner N, Wang Y, Clarke R. Association of interferon regulatory factor-1, nucleophosmin, nuclear factor-kappaB, and cyclic AMP response element binding with acquired resistance to faslodex (ICI 182,780). Cancer Res. 2002;62:3428–37.
35. Pratt MAC, Bishop TE, White D, Yasvinski G, Menard M, Niu MY, Clarke R. Estrogen withdrawal-induced NF-kappaB activity and BCL-3 expression in breast cancer cells: roles in growth and hormone independence. Mol Cell Biol. 2003;23:6887–900.
36. Riggins R, Zwart A, Nehra R, Agarwal P, Clarke R. The NFkB inhibitor parthenolide restores ICI 182,780 (Faslodex; Fulvestrant)-induced apoptosis in antiestrogen resistant breast cancer cells. Mol Cancer Ther. 2005;4:33–41.
37. Ning Y, Riggins R, Mulla JE, Chung H, Zwart A, Clarke R. Interferon gamma restores breast cancer sensitivity to Fulvestrant by regulating STAT1, IRF1, NFkB, BCL2 family members and signaling to a caspase-dependent apoptosis. Mol Cancer Ther. 2010;9:1274–85.
38. Crawford AC, Riggins R, Shajahan AN, Zwart A, Clarke R. Co-inhibition of BCL-W and BCL2 restores antiestrogen sensitivity through BECN1 and promotes an autophagy-associated necrosis. PLoS One. 2010;5:e8604.
39. Cook KL, Clarke PAG, Parmar J, Hu R, Schwartz-Roberts JL, Abu-Asab M, Wärri A, Baumann WT, Clarke R. Knockdown of estrogen receptor alpha induces autophagy and inhibits antiestrogen-mediated unfolded protein response activation promoting ROS-induced breast cancer cell death. FASEB J. 2014;28:3891–905.
40. Nehra R, Riggins R, Shajahan AN, Zwart A, Crawford AC, Clarke R. BCL2 and CASP8 regulation by NFkB differentially affect mitochondrial function and cell fate in antiestrogen sensitive and resistant breast cancer cells. FASEB J. 2010;24:2039–54.
41. Bouker KB, Skaar TC, Fernandez DR, O'Brien KA, Clarke R. Interferon regulatory factor-1 mediates the proapoptotic but not cell cycle arrest effects of the steroidal antiestrogen ICI 182,780 (Faslodex, Fulvestrant). Cancer Res. 2004;64:4030–9.
42. Bouker KB, Skaar TC, Riggins R, Harburger DS, Fernandez DR, Zwart A, Wang A, Clarke R. Interferon regulatory factor-1 (IRF-1) exhibits tumor suppressor activities in breast cancer associated with caspase activation and induction of apoptosis. Carcinogenesis. 2005;26:1527–35.
43. Zhu Y, Singh B, Hewitt S, Liu A, Gomez B, Wang A, Clarke R. Expression patterns among interferon regulatory factor-1, human X-box binding protein-1, nuclear factor kappa B, nucleophosmin, estrogen receptor alpha and progesterone receptor proteins in breast cancer tissue microarrays. Int J Oncol. 2006;28:67–76.

44. Bouker KB, Skaar TC, Harburger DS, Riggins R, Fernandez DR, Zwart A, Clarke R. The A4396G polymorphism in interferon regulatory factor-1 is frequently expressed in breast cancer. Cancer Genet Cytogenet. 2007;175:61–4.
45. Schwartz-Roberts JL, Cook KL, Chen C, Shajahan-Haq AN, Axelrod M, Warri A, RIggins RB, Jin L, Haddad BR, Kallakury BV, Baumann WT, Clarke R. Interferon regulatory factor-1 signaling regulates the switch between autophagy and apoptosis to determine breast cancer cell fate. Cancer Res. 2015;75:1046–55.
46. Gomez BP, Riggins R, Shajahan AN, Klimach U, Wang A, Crawford AC, Zhu Y, Zwart A, Wang M, Clarke R. Human X-Box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. FASEB J. 2007;21:4013–27.
47. Shajahan-Haq AN, Cook KL, Schwartz-Roberts JL, Eltayeb AE, Demas DM, Warri AM, Facey CO, Hilakivi-Clarke LA, Clarke R. MYC regulates the unfolded protein response and glucose and glutamine uptake in endocrine resistant breast cancer. Mol Cancer. 2014;13:239.
48. Hu R, Warri A, Jin L, Zwart A, Riggins R, Clarke R. NFkappaB signaling is required for XBP1 (U and S) mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol. 2015;35:390.
49. Skaar TC, Prasad SC, Sharareh S, Lippman ME, Brunner N, Clarke R. Two-dimensional gel electrophoresis analyses identify nucleophosmin as an estrogen regulated protein associated with acquired estrogen- independence in human breast cancer cells. J Steroid Biochem Mol Biol. 1998;67:391–402.
50. Zhang YW, Nasto RE, Varghese R, Jablonski SA, Serebriiskii IG, Surana R, Calvert VS, Bebu I, Murray J, Jin L, Johnson M, Riggins R, Ressom H, Petricoin E, Clarke R, Golemis EA, Weiner LM. Acquisition of estrogen independence induces TOB1-related mechanisms supporting breast cancer cell proliferation. Oncogene. 2016;35:1643–56.
51. Shajahan AN, Riggins R, Clarke R. The role of X-box binding protein-1 in tumorigenicity. Drug News Perspect. 2009;22:241–6.
52. Piccart M, Hortobagyi GN, Campone M, Pritchard KI, Lebrun F, Ito Y, Noguchi S, Perez A, Rugo HS, Deleu I, Burris HAIII, Provencher L, Neven P, Gnant M, Shtivelband M, Wu C, Fan J, Feng W, Taran T, Baselga J. Everolimus plus exemestane for hormone-receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: overall survival results from BOLERO-2. Ann Oncol. 2014;25:2357–62.
53. Hortobagyi GN. Everolimus plus exemestane for the treatment of advanced breast cancer: a review of subanalyses from BOLERO-2. Neoplasia. 2015;17:279–88.
54. Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, Iwata H, Harbeck N, Zhang K, Theall KP, Jiang Y, Bartlett CH, Koehler M, Slamon D. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol. 2016;17(4):425–39.
55. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, Harbeck N, Lipatov ON, Walshe JM, Moulder S, Gauthier E, Lu DR, Randolph S, Dieras V, Slamon DJ. Palbociclib and Letrozole in advanced breast cancer. N Engl J Med. 2016;375:1925–36.
56. Verma S, Bartlett CH, Schnell P, DeMichele AM, Loi S, Ro J, Colleoni M, Iwata H, Harbeck N, Cristofanilli M, Zhang K, Thiele A, Turner NC, Rugo HS. Palbociclib in combination with Fulvestrant in women with hormone receptor-positive/HER2-negative advanced metastatic breast cancer: detailed safety analysis from a multicenter, randomized, placebo-controlled, phase III study (PALOMA-3). Oncologist. 2016;21:1165–75.
57. Rugo HS. Dosing and safety implications for oncologists when administering everolimus to patients with hormone receptor-positive breast cancer. Clin Breast Cancer. 2016;16:18–22.
58. Dominicus CS, Patel V, Chambers JE, Malzer E, Marciniak SJ. Endoplasmic reticulum stress signaling during development. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.

59. Morreal J, Hong F, Li Z. Regulation of the unfolded protein response and its roles in tumorigenesis and cancer therapy. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
60. Clarke R, Cook KL, Hu R, Facey CO, Tavassoly I, Schwartz JL, Baumann WT, Tyson JJ, Xuan J, Wang Y, Warri A, Shajahan AN. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. Cancer Res. 2012;72:1321–31.
61. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep. 2006;7:880–5.
62. Lee AS. The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci. 2001;26:504–10.
63. Jovaisaite V, Auwerx J. The mitochondrial unfolded protein response-synchronizing genomes. Curr Opin Cell Biol. 2015;33:74–81.
64. Vihervaara A, Duarte FM, Lis JT. Molecular mechanisms driving transcriptional stress responses. Nat Rev Genet. 2018;19:385–97.
65. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334:1081–6.
66. Livezey M, Kim JE, Shapiro DJ. A new role for estrogen receptor alpha in cell proliferation and cancer: activating the anticipatory unfolded protein response. Front Endocrinol. 2018;9:325.
67. Clarke R. Introduction: the unfolded protein response. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
68. Li C, Fan Q, Quan H, Nie M, Luo Y, Wang L. The three branches of the unfolded protein response exhibit differential significance in breast cancer growth and stemness. Exp Cell Res. 2018;367:170–85.
69. Zhang F, Hamanaka RB, Bobrovnikova-Marjon E, Gordan JD, Dai MS, Lu H, Simon MC, Diehl JA. Ribosomal stress couples the unfolded protein response to p53-dependent cell cycle arrest. J Biol Chem. 2006;281:30036–45.
70. Chen X, Shen J, Prywes R. The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi. J Biol Chem. 2002;277:13045–52.
71. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, Goldstein JL. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell. 2000;6:1355–64.
72. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M, Mori K. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol Cell Biol. 2000;20:6755–67.
73. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001;107:881–91.
74. Clauss IM, Chu M, Zhao JL, Glimcher LH. The basic domain/leucine zipper protein hXBP-1 preferentially binds to and transactivates CRE-like sequences containing an ACGT core. Nucleic Acids Res. 1996;24:1855–64.
75. Park KK, Jung E, Chon SK, Seo M, Kim HW, Park T. Finding of TRE (TPA responsive element) in the sequence of human taurine transporter promoter. Adv Exp Med Biol. 2003;526:159–66.
76. Liou HC, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik L, Ting JP, Glimcher LH. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. Science. 1990;247:1581–4.
77. Clauss IM, Gravallese EM, Darling JM, Shapiro F, Glimcher MJ, Glimcher LH. In situ hybridization studies suggest a role for the basic region- leucine zipper protein hXBP-1 in exocrine gland and skeletal development during mouse embryogenesis. Dev Dyn. 1993;197:146–56.

78. Zhang Z, Hildebrandt EF, Simbulan-Rosenthal CM, Anderson MG. Sequence-specific binding of poly(ADP-ribose) polymerase-1 to the human T cell leukemia virus type-I tax responsive element. Virology. 2002;296:107–16.
79. Cox JM, Sloan LS, Schepartz A. Conformation of Tax-response elements in the human T-cell leukemia virus type I promoter. Chem Biol. 1995;2:819–26.
80. Fujita E, Kouroku Y, Isoai A, Kumagai H, Misutani A, Matsuda C, Hayashi YK, Momoi T. Two endoplasmic reticulum-associated degradation (ERAD) systems for the novel variant of the mutant dysferlin: ubiquitin/proteasome ERAD(I) and autophagy/lysosome ERAD(II). Hum Mol Genet. 2007;16:618–29.
81. Tyson JJ, Baumann WT, Chen C, Verdugo A, Tavassoly I, Wang Y, Weiner LM, Clarke R. Dynamic modeling of oestrogen signalling and cell fate in breast cancer cells. Nat Rev Cancer. 2011;11:523–32.
82. Hu R, Clarke R. Roles of spliced and unspliced XBP1 in breast cancer. In: Clarke R, editor. Introduction to the unfolded protein response. New York: Springer; 2018.
83. Dickson RB, Lippman ME. Growth factors in breast cancer. Endocr Rev. 1995;16:559–89.
84. Lowry MC, Gallagher WM, O'Driscoll L. The role of exosomes in breast cancer. Clin Chem. 2015;61:1457–65.
85. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013;501:346–54.
86. Marusyk A, Tabassum DP, Altrock PM, Almendro V, Michor F, Polyak K. Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. Nature. 2014;514(7520):54–8.
87. Mani NL, Schalper KA, Hatzis C, Saglam O, Tavassoli F, Butler M, Chagpar AB, Pusztai L, Rimm DL. Quantitative assessment of the spatial heterogeneity of tumor-infiltrating lymphocytes in breast cancer. Breast Cancer Res. 2016;18:78.
88. Clarke R, Tyson JJ, Tan M, Baumann WT, Xuan J, Wang Y. Systems biology: perspectives on multiscale modeling in research on endocrine-related cancers. Endocr Relat Cancer. 2018; in revision.
89. Parmar JH, Cook KL, Shajahan-Haq AN, Clarke PA, Tavassoly I, Clarke R, Tyson JJ, Baumann WT. Modelling the effect of GRP78 on anti-oestrogen sensitivity and resistance in breast cancer. Interface Focus. 2013;3:20130012.
90. Tavassoly I, Parmar J, Shajahan-Haq AN, Clarke R, Baumann WT, Tyson JJ. Dynamic modeling of the interaction between autophagy and apoptosis in mammalian cells. CPT Pharmacometrics Syst Pharmacol. 2015;4:263–72.
91. Chen L, Xuan J, Riggins R, Wang Y, Hoffman EP, Clarke R. Multilevel support vector regression analysis to identify condition-specific regulatory networks. Bioinformatics. 2010;26:1416–22.
92. Zhang B, Li H, Riggins R, Zhan M, Xuan J, Zhang Z, Hoffman EP, Clarke R, Wang Y. Differential dependency network analysis to identify condition-specific topological changes in biological networks. Bioinformatics. 2009;25:526–32.
93. Erlich S, Mizrachy L, Segev O, Lindenboim L, Zmira O, di-Harel S, Hirsch JA, Stein R, Pinkas-Kramarski R. Differential interactions between Beclin 1 and Bcl-2 family members. Autophagy. 2007;3:561–8.
94. Lindqvist LM, Vaux DL. BCL2 and related prosurvival proteins require BAK1 and BAX to affect autophagy. Autophagy. 2014;10:1474–5.
95. Tian S, Lin J, Jun ZJ, Wang X, Li Y, Ren X, Yu W, Zhong W, Xiao J, Sheng F, Chen Y, Jin C, Li S, Zheng Z, Xia B. Beclin 1-independent autophagy induced by a Bcl-XL/Bcl-2 targeting compound, Z18. Autophagy. 2010;6:1032–41.
96. Hilakivi-Clarke LA, Wärri A, Bouker KB, Zhang X, Cook KL, Jin L, Zwart A, Nguyen N, Hu R, Cruz MI, de Assis S, Wang X, Xuan J, Wang Y, Wehrenberg B, Clarke R. Effects of *in utero* exposure to ethinyl estradiol on tamoxifen resistance and breast cancer recurerence in a preclinical model. J Natl Cancer Inst. 2017;109:1–11.

97. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, HUng MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res. 2008;68:6084–91.
98. Murphy LC, Skliris GP, Rowan BG, Al-Dhaheri M, Williams C, Penner C, Troup S, Begic S, Parisien M, Watson PH. The relevance of phosphorylated forms of estrogen receptor in human breast cancer in vivo. J Steroid Biochem Mol Biol. 2009;114:90–5.
99. Anbalagan M, Rowan BG. Estrogen receptor alpha phosphorylation and its functional impact in human breast cancer. Mol Cell Endocrinol. 2015;418(Pt 3):264–72.
100. Katzenellenbogen JA, Mayne CG, Katzenellenbogen BS, Greene GL, Chandarlapaty S. Structural underpinnings of oestrogen receptor mutations in endocrine therapy resistance. Nat Rev Cancer. 2018;18:377–88.
101. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, Majumder PK, Baselga J, Rosen N. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell. 2011;19:58–71.
102. Warri A, Cook KL, Hu R, Jin L, Zwart A, Soto-Pantoja DR, Liu J, Finkel T, Clarke R. Autophagy and unfolded protein response (UPR) signaling regulate progression of mammary gland involution by restraining apoptosis-driven irreversible changes. Cell Death Discovery. 2018;5:40.
103. Clarke R, Shajahan AN, Wang Y, Tyson JJ, Riggins R, Weiner LM, Baumann WT, Xuan J, Zhang B, Facey C, Aiyer H, Cook K, Hickman FE, Tavassoly I, Verdugo A, Chen C, Zwart A, Wärri A, Hilakivi-Clarke LA. Endoplasmic reticulum stress, the unfolded protein response, and gene network modeling in antiestrogen resistant breast cancer. Horm Mol Biol Clin Invest. 2011;5:35–44.
104. Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer. 2014;14:263–76.
105. Hollien J. Evolution of the unfolded protein response. Biochim Biophys Acta. 2013;1833:2458–63.

Chapter 9
Outside the Endoplasmic Reticulum: Non-Canonical GRP78 Signaling

Katherine L. Cook

Abstract Glucose-regulated protein 78 is best known for its protein chaperone activities and as acting as a molecular switch controlling the unfolded protein response signaling pathway within the endoplasmic reticulum. GRP78 binds to the three arms of the unfolded protein response rendering each pathway inactive. In the presence of unfolded or misfolded proteins, GRP78 unbinds from the three signaling arms, thus enabling UPR activation. However, GRP78 is ubiquitously localized within other cellular compartments and has varied molecular signaling functions depending upon its localization. GRP78 is found on the cell membranes to control cellular growth and differentiation pathways, GRP78 is found on mitochondria, GRP78 regulates cellular energetic pathways and metabolism, GRP78 controls apoptosis and cell death pathway signaling, GRP78 modulates autophagy, and GRP78 affects immune cell activities. We will review the pleiotropic activities of GRP78 outside of its normal UPR functions and discuss GRP78 signaling in cancer in this book chapter.

Keywords Breast cancer · Obesity · Therapeutic resistance · Autophagy · Mitochondria · Metabolism · Immunity · Macrophage · CD47

GRP78, Protein Chaperone of the Unfolded Protein Response

The unfolded protein response (UPR) is an endoplasmic reticulum (EnR) stress pathway activated when the cell undergoes certain stressors that result in the accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum. Hypoxia, nutrient deprivation, genetic mutation resulting in protein aggregates, and certain drugs can activate UPR [1]. Initially, the overall goal of UPR

K. L. Cook (✉)
Department of Surgery and Comprehensive Cancer Center,
Wake Forest University School of Medicine, Winston-Salem, NC, USA
e-mail: klcook@wakehealth.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_9

is cytoprotective by correcting the protein folding and reducing protein load in the endoplasmic reticulum; however, an extended duration of UPR signaling promotes cell death. The three UPR signaling arms include PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). These signaling molecules are held inactive in the EnR through binding with glucose-regulated protein 78 (GRP78). In the presence of unfolded proteins, GRP78 unbinds enabling UPR activation. PERK phosphorylates eIF2α resulting in the halt of cap-dependent protein translation and induces ATF4 transcription. Extended PERK signaling induces pro-apoptotic CHOP (DDIT3) transcription to promote cell death. IRE1 activation results in both endonuclease and kinase activities. IRE1 unconventionally splices a 26 base pair intron out of X-box binding protein 1 (XBP1) mRNA in the cytosol resulting in the highly active transcription factor XBP1-S. Once activated, IRE1 also phosphorylates the mitogen activated protein kinase (MAPK) c-Jun terminal kinase (JNK). Elevated JNK phosphorylation can promote cell death. Unbound from GRP78, ATF6 translocates from the EnR to the Golgi complex where it is cleaved by site 1 and site 2 proteases to form a highly active transcription factor, to promote the transcription of protein chaperones (GRP78 and GRP94) and XBP1, hence feeding back into the UPR system [1].

Glucose-regulated protein 78 (GRP78), also known as binding immunoglobulin protein (BiP) or heat-shock protein family A, member 5 (HSPA5) was originally independently characterized as a glucose deprivation-sensitive broad specificity molecular chaperone localized to the EnR [2, 3]. GRP78 is a member of the heat-shock protein 70 subfamily and contains two major domains, an N-terminal ATPase domain and C-terminal peptide substrate binding domain. GRP78 chaperone activities are therefore dependent on cellular energetic capacity [4]. GRP78 biological activities are multifunctional. GRP78 facilitates folding and assembly of nascent peptides, reduces peptide misfolding, prevents polypeptide aggregation, targets misfolded proteins for proteasome degradation, and acts as a molecular switch for UPR signaling [5, 6]. While GRP78 is most known for these EnR activities, GRP78 pleiotropic functions outside the EnR participate in a wide variety of cell signaling processes and will be further discussed in the following sections.

GRP78 in Breast Cancer and Therapeutic Responsiveness

The unfolded protein response signaling components are often found upregulated in cancer. Elevated XBP1 is found in human breast cancer tumors and is associated with hypoxia and aggressiveness [7–10]. Overexpression of GRP78 is reported in breast cancer cell lines and in malignant breast tumors but not benign breast lesions [11–13]. Moreover, elevated XBP1 and GRP78 expression correlates with endocrine therapy resistance in ER+ breast cancer [1, 9, 11]. Overexpression of GRP78 in MCF-7 and LCC1 tamoxifen sensitive breast cancer cell lines induced

therapeutic resistance to antiestrogen therapies [11]. Overexpression of GRP78 in MCF7-BUS cells (an ER+ estrogen dependent breast cancer cell line) resulted in the inhibition of estrogen deprivation-induced apoptosis, implicating elevated GRP78 expression in aromatase inhibitor resistance [14]. In a rat DMBA model of mammary carcinogenesis treated with tamoxifen, tumors that initially responded to antiestrogen therapy then lost therapeutic efficacy over the course of treatment (acquired resistance) had elevated GRP78 protein levels when compared with tumors from untreated rats, tumors that responded to therapy (complete response), or tumors that never responded to tamoxifen (de novo resistance), giving further evidence of the impact of GRP78 overexpression in promoting therapeutic resistance [11]. Targeting either XBP1 or GRP78 was sufficient to restore endocrine-targeting therapy responsiveness in resistant ER+ breast cancer cell lines [9, 11]. Furthermore, targeting GRP78 in vivo using an antisense morpholino restored tamoxifen sensitivity in resistant ER+ tumors and potentiated therapeutic responsiveness in tamoxifen sensitive tumors [15]. Inhibition of GRP78 reduced tumorigenesis in a spontaneous MMTV-induced breast cancer model. GRP78 heterozygosity correlated with reduced micro-vascular density and prevented endothelial cell migration, implicated GRP78 targeting as an anti-angiogenic therapy [16, 17]. Others have shown elevated GRP78 reduces relapse-free survival after Adriamycin-based adjuvant chemotherapy for the treatment of breast cancer, implicating GRP78 in doxorubicin resistance [18]. Furthermore, GRP78 overexpression attenuated gemcitabine chemosensitivity in breast cancer cells through modulation of pro-oncogenic AKT signaling [19]. Interestingly, in patients that received taxane therapy the opposite effect was observed; GRP78 expression correlated with a positive response to adjuvant taxane-based chemotherapy in breast cancer patients [20]. These data suggest that GRP78 regulation of therapeutic responsiveness is drug mechanism dependent.

GRP78 Regulates Apoptosis

With the bulk of literature implicating GRP78 in the development of therapeutic resistance, the link between GRP78 and cell death pathways (such as apoptosis) has been investigated. Transmembrane EnR GRP78 can localize with the effector caspase, pro-caspase-7 and prevent activation of the apoptotic cascade. Mutation of the ATP binding domain of GRP78 prevented GRP78 binding to procaspase-7, thereby losing the protective effect of GRP78 overexpression on mediating etoposide resistance [21]. Knockdown of GRP78 in endocrine therapy resistant breast cancer cell lines expressed high levels of cleaved caspase-7 and cleaved PARP (activated form), demonstrating upregulation of apoptosis mediated by GRP78 targeting [11]. Moreover, overexpression of GRP78 in endocrine therapy sensitive breast cancer cell lines inhibited therapy-induced cleavage of caspase-7 and PARP, preventing the activation of apoptosis [11]. Outer surface EnR GRP78 was also shown to interact

with pro-apoptotic Bik and antiapoptotic BCL2 mitochondrial apoptosis regulating proteins. GRP78 can sequester Bik, preventing Bik-mediated release of BCL2 preventing and blocking mitochondrial-induced apoptotic signaling [14, 22]. Supporting the antiapoptotic role of GRP78, knockout of GRP78 promoted tissue caspase cascade and cell death [23, 24]. Moreover, in cancer models knockdown of GRP78 impeded tumor growth and elevated tumor apoptosis, clearly demonstrating the link between GRP78 and apoptosis [16, 23, 25, 26].

GRP78 Controls Autophagy

Autophagy is a cellular pathway of "self-eating" whereby a cell can digest and recycle old or damaged organelles and proteins. This process involves the formation of a LC3-positive double membrane vesicle around cellular cargo, lysosomal degradation of the cargo, and the release of the recycled nutrients from the autophagosome to fuel metabolism. Autophagy is often found upregulated in many solid tumors and promotes therapeutic resistance [1, 27]. Targeting autophagy was shown to restore therapeutic sensitivity and promote therapeutic responsiveness [28, 29]. Since the initial goal of UPR activation is restoration of homeostasis, it is biologically pragmatic to integrate UPR signaling with autophagy as a mechanism to clear misfolded proteins. UPR pathway intersects with autophagic signaling at various stages: PERK activation of ATF4 promotes the transcription of autophagy-related gene 12 (ATG12) [27], IRE1-mediated JNK activation modulates autophagic signaling, unspliced XBP1 (XBP1-U) controls autophagy through regulation of FoXO1 [30], and ATF6-mediated regulation of autophagy critical protein death-associated protein kinase-1 [31]. Literature also indicates a critical role of GRP78 in mediating autophagic signaling independent of its UPR and protein chaperone activities. Ovarian cancer HeLa cells were transfected with scrambled control or GRP78 siRNA and treated with tunicamycin, an inhibitor of N-glycosylation resulting in the accumulation of misfolded proteins in the EnR, to induce UPR signaling. GRP78 knockdown prevented EnR stress-induced LC3-GFP puncta formation [32]. It was also shown that GRP78 inhibition prevention of autophagy was independent of PI3K and beclin-1 activities [32]. GRP78 was also shown to be critical for the induction of pro-survival endocrine therapy-induced autophagy in ER+ breast cancer [11, 33]. Targeting GRP78 regulated AMPK activity to control mTOR-regulated suppression of autophagy [11, 33]. The regulation of the AMPK/mTOR signaling axis by GRP78 to control autophagy induction was also observed in rat pheochromocytoma (PC12) cells [34]. Moreover, overexpression of GRP78 led to elevated autophagy-related gene 9 (ATG9) protein levels [11], giving further support to the link between GRP78 and autophagy. Others showed in myeloma that GRP78 enhances protein aggregate incorporation into the autophagosomes, further supporting the crossover role of GRP78 regulating autophagy [35].

GRP78 on the Cell Surface

It was reported that sub-fraction of the cellular GRP78 protein load can localize to the plasma membrane in cancer cells [36]. While cell surface GRP78 levels are detectable in unstressed conditions in normal cells, EnR stress was shown to increase cell surface GRP78 expression [36]. Deletion of the GRP78 KDEL EnR targeting sequence prevents EnR stress-induced cell surface localization of GRP78. Taken together, these data suggest that stress-induced protein chaperone induction may overwhelm the KDEL EnR targeting machinery allowing a "leaky" phenotype enabling increased cell surface expression of GRP78. Due to the highly proliferative nature of cancer cells requiring an elevated EnR capacity for protein synthesis and folding, "leaky" KDEL targeting may inherently enhance cancer cell surface GRP78 levels. In support of this hypothesis, analysis of the plasma membrane proteome in A549 lung cancer cells identified an abundance of chaperone proteins including GRP78 [37]. Moreover, data demonstrated that cell surface GRP78 exists as a peripheral protein through its interaction with GPI-anchored proteins and other cell surface proteins [38]. While the function of cell surface GRP78 is still being elucidated, emerging studies highlight the important role of cell surface GRP78 in signal transduction.

Hormone resistant breast and prostate cancer cell lines expressed elevated cell surface GRP78. Moreover, EnR stress inducing agents increased GRP78 localization to the cell membrane. Cell surface GRP78 was able to complex with PI3K signaling to promote PIP3 formation. Mutation of GRP78 at the N-terminus region resulted in decreased co-localization of cell surface GRP78 with the p85 subunit of PI3K, resulting in the inhibition of PIP3 generation [39]. Targeting cell surface GRP78 would then inhibit PI3K signaling to suppress therapeutic resistance.

Cell surface localization of GRP78 interacts with various signaling molecules to induce proliferation and promote the metastatic cascade. Localization of GRP78 to the cell membrane stimulates breast cancer cell proliferation and increases metastatic potential through regulation of STAT3 signaling [40]. Moreover, GRP78 on the cell surface was also shown to interact with α_2-macroglobulin to promote growth and metastasis in prostate cancer cells [41]. GRP78 was also shown to complex with teratocarcinoma-derived growth factor-1 (Cripto-1) on the cell membrane to control transforming growth factor- β (TGF-β) signaling and proliferation [42]. Moreover, Cripto and GRP78 are highly expressed in metastatic prostate cancer and co-expression of both Cripto and GRP78 correlates with poor survival in prostate cancer [43]. The regulation of TGF-β by GRP78 may result in tumor initiation and represent an early stage of tumorigenesis regulated by EnR stress.

The regulation of GRP78 on the cell surface may not only affect cell proliferative signaling pathways but also may modulate apoptosis. Induction of EnR stress by tunicamycin or treatment with cytotoxic chemotherapeutic agent, doxorubicin, resulted in the recruitment of GRP78 to the cell membrane in triple-negative breast cancer cells. The blockade of cell surface GRP78 by an anti-GRP78 antibody

prevented CHOP-mediated apoptosis and drug-induced cell death [44]. Others showed that cell surface GRP78 on endothelial cell interacts with tumoral Kringle 5 to mediate the anti-angiogenic and pro-apoptotic activities when exposed to hypoxic or cytotoxic stressed cancer cell [45, 46]. Furthermore, analysis of cell surface GRP78 protein levels in human breast tumors early stage operable patients, in patients before systemic neoadjuvant chemotherapy, and in patients following neoadjuvant chemotherapy indicated that cell surface GRP78 was a potential positive prognostic marker of response to chemotherapy and improved disease-free survival [47]. These data suggest that cell surface GRP78 localization results in multifaceted and sometimes opposing signaling induction that may be context (cell type, drug, or stressor specific) dependent.

Secreted GRP78 Function

In many solid tumors, including breast, gastric, prostate, and colon cancer, GRP78 can be secreted into the microenvironment and may play an important role in mediating drug resistance [48–51]. Secreted GRP78 inhibited the anti-angiogenic activities of bortezomib [52]. Secreted GRP78 was shown to activate epidermal growth factor receptor (EGFR) signaling in hepatocellular carcinoma cell lines to promote resistance to sorafenib [50]. Moreover, secreted GRP78 promoted differentiation of bone marrow mesenchymal stem cells to cancer-associated fibroblasts to facilitate tumor stability and growth [53]. Therefore, secreted GRP78 may enhance tumor growth and facilitate drug resistance.

GRP78 on the Mitochondria

It was demonstrated that while GRP78 generally localizes to the EnR in quiescent cells and is associated with other EnR markers such as calnexin and protein disulfide-isomerase, when cells are treated with a calcium ionophore (A23187) or a EnR Ca^{2+}-ATPase inhibitor (thapsigargin) to induce UPR signaling GRP78 localization shifts to the mitochondria [54]. In sub-mitochondrial fractionation studies, EnR stress promoted GRP78 localization to the mitochondria intermembrane space, inner membrane, and matrix, but not the outer membrane [54]. The cellular function of mitochondrial GRP78 localization under EnR stress is still under investigation. However, it can be speculated that under EnR stress, mitochondrial GRP78 localization may promote cell survival through mitochondrial membrane interactions and may mediate pro-survival mitochondrial bioenergetics. Indeed, others demonstrated that under EnR stress, GRP78 retrotranslocation from the EnR to the mitochondria is associated with clusterin co-trafficking and stabilizing the mitochondrial membrane integrity to reduce stress-induced apoptosis [55]. Further studies indicate that

mitochondrial GRP78 plays an integral role in stabilizing AAA domain containing 3A (ATAD3A)/WASF3 tumor metastasis promoting complex at the mitochondrial membrane [56], giving evidence supporting pro-survival signaling of mitochondrial GRP78 localization.

GRP78 Regulates Cellular and Mitochondrial Metabolism

Recent work has demonstrated a novel role of GRP78 in regulating lipid metabolism. In a metabolomics analysis, knockdown of GRP78 in breast cancer cells resulted in the accumulation of polyunsaturated fatty acid metabolites [15]. Inhibition of GRP78 modulated AMPK activity, reduced phosphorylation of acetyl-coA carboxylase, and inhibited mitochondrial carnitine palmitoyltransferase 1A (CPT1A) protein levels [11, 15]. GRP78-regulated CPT1A inhibition prevented mitochondrial import of fatty acid metabolites to serve as substrates for β-oxidation, resulting in decreased overall cellular bioenergetics and an accumulation of fatty acid metabolites in the cytosol. The elevated cytosolic lipid metabolites served as a catalyst for elevated lipid peroxidation and reactive oxygen species generation promoting cell death [15]. GRP78-mediated regulation of β-oxidation may then result in a shift of mitochondria bioenergetics. This was recently shown in Soto-Pantoja et al., where targeting GRP78 in macrophages resulted in a bioenergetic shift effecting macrophage polarity [57]. Transfection of mouse macrophage cell line RAW 264.7 with GRP78 siRNA elevated cellular lipid content accumulation, reduced mitochondrial oxygen consumption rates, and reduced basal extracellular acidification rate [57].

Inhibition of GRP78 also regulated sterol regulatory element binding protein-1 and -2 (SREBP1/SREBP2) transcription and some of the downstream target genes [15]. SREBP1 is a master regulator of glucose metabolism and lipogenesis, while SREBP2 modulates cholesterol biogenesis. SREBP1 modulates fatty acid synthase (FASN), ACSL, and SCD1 transcription. SREBP1 and SREBP2 are held inactive in the EnR membrane, similarly to ATF6, and translocate to the Golgi complex to be cleaved by site1/site2 proteases for full activation. Overexpression of GRP78 was shown to sequester SREBP1 and -2 to the EnR preventing translocation and thereby inhibiting SREBP1 and -2 activities [58]. However, GRP78 silencing was also shown to inhibit SREBP1 and -2 activation by reducing the protein SCAP which is necessary for SREBP1 and -2 packaging and transport to the Golgi complex [15]. Therefore, both overexpression and inhibition of GRP78 may affect lipogenesis through inhibition of SREBP1 and SREBP2.

Direct physical interaction between endoplasmic reticulum membranes and mitochondrial outer membranes was shown to effect mitochondrial biological function [59]. The creation of an EnR-mitochondrial signaling axis enables cross-talk between stress and metabolism. Recently, mitochondrial-associated EnR membrane localization of GRP78 was shown necessary for the correct folding of the

mitochondria-localized steroidogenic acute regulatory protein (StAR) [60]. StAR is required for cholesterol transport into the mitochondrial to serve as the building blocks for steroidogenesis. StAR absence is lethal: inhibition of StAR prevents steroid synthesis resulting in salt imbalance and death [61]. Knockdown of GRP78 reduced StAR protein levels and activity [60], giving further evidence of interplay between GRP78 and mitochondrial metabolic pathways.

Obesity-Mediated Breast Cancer, UPR, and Immunity

Over 2.1 billion people worldwide are considered overweight or obese [62]. Obesity also is an epidemic in the USA, as over 60% of women are overweight or obese (a body mass index of over 30). Several studies have demonstrated a strong link between obesity and a greater risk of developing breast cancer by as much as 50% in postmenopausal women [63, 64]. Moreover, obesity is associated with worse overall survival in TNBC premenopausal women [65–67]. Studies have reported a threefold higher breast cancer mortality rate in obese women and it is estimated that 3 out of 10 breast cancers may have been prevented if the women were not overweight, indicating an important role for obesity in the etiology of breast cancer [63]. Results from the Breast International Group (BIG) I-98 study indicate that obese women treated with tamoxifen had a poorer overall survival when compared to healthy weight women, implicating a causal link between obesity and endocrine therapy resistance [68]. However, loss/mutation of ERα expression only occurs in a minority (15–20%) of resistant breast cancers, suggesting regulation of other key molecular signaling pathways as the predominate driver of endocrine therapy resistance [69, 70].

Obesity is also a predictor of poor response to chemotherapy. Other studies investigating the impact of obesity on women breast cancer patients receiving neoadjuvant anthracycline-based chemotherapy indicated higher BMI was associated with worse pathological complete response. Moreover, obese patients were more likely to have hormone negative tumors, implicating obesity in both TNBC tumorigenesis and the development of doxorubicin resistance [65, 71, 72].

Literature indicates a possible role of increased circulating estradiol (which would not easily explain increased TNBC risk), elevated leptin, and modulated insulin growth factor signaling as molecular mechanism(s) of obesity-mediated breast cancer risk, albeit these signaling pathways only explain in part how obesity promotes tumorigenesis [73, 74]. We previously showed that diet and obesity impact UPR signaling components in a murine DMBA model [75]. Consumption of a high fat diet elevated GRP78 protein levels in mammary glands and tumors of female C57/Bl6 mice, implicating UPR signaling as a possible driver of obesity-mediated breast cancer. Literature also demonstrated that obesity upregulated GRP78 protein levels in the subcutaneous and visceral fat of obese diabetic human patients [76]. Elevated GRP78 expression in adipocytes predicted endometrial cancer progression and patient survival [77], suggesting endoplasmic reticulum stress and GRP78 protein levels in fat deposits as a driver of cancer progression. Moreover, waist circumfer-

ence was positively correlated with ATF6 and GRP78 expression in circulating immune cells [78]. Our group recently showed that UPR signaling differentially affects macrophage polarity to modulate breast cancer cell clearance and immune checkpoint therapy responsiveness [57]. Taken together these data suggest that obesity may elevate immune cell UPR signaling to promote dysfunction and reduce immunosurveillance enabling tumor survival and drug resistance. Therefore, obesity may directly stimulate EnR stress to elevate GRP78 protein levels to predispose tumors to therapeutic resistance or obesity may indirectly promote cancer cell survival by preprograming immune cells enabling avoidance of immune cell detection.

GRP78/CD47 Signaling Axis Regulates Immunity

CD47 is a widely expressed receptor that regulates phagocytic activity when it engages its counter-receptor, SIRPα, on macrophages. During normal homeostatic conditions, expression of CD47 on erythrocytes prevents their phagocytosis for which this receptor is known as a "don't eat me" signal [79]. CD47 overexpression is associated with poor prognosis in cancer. This may be due, in part, as the expression of CD47 receptor allows bypassing of innate immune mechanisms to clear tumor cells [80]. CD47 also acts as a receptor to its ligand thrombospondin-1 (TSP1). Binding of TSP1 to CD47 profoundly inhibits the nitric oxide (NO) signaling pathway affecting cell survival and many physiological functions [80].

Studies previously demonstrated in cancer models that the regulation of CD47's phagocytic activity results with the counter-regulation of "eat me" signals such as the EnR localized protein, calreticulin [81]. Blockade with anti-human CD47 antibody in cancer cells enhances phagocytic activity by the regulation of calreticulin and pro-immunological cell death protein high mobility box group 1 (HMBG1) [81]. UPR signaling was previously implicated in the regulation and processing of calreticulin, but until recently a connection to CD47 was not clear. We demonstrated that targeting GRP78 re-established sensitivity to antiestrogen therapy in a murine model of breast cancer [15]. This was associated with a regulation of lipid metabolism, downregulation of CD47, and upregulation of HMGB1 gene expression. Moreover, we observed increased macrophage infiltration to the tumor. This was the first instance of a connection between CD47 and the UPR pathway [15]. Recent studies correlate CD47 and GRP78 expression with reduced relapse-free survival in breast cancer patients, particularly in the development of endocrine therapy resistance, where co-expression of GRP78 and CD47 is positively correlated with recurrence in ER+ breast cancer patients treated with endocrine-targeting therapies [82]. Expression of CD47 alone was not associated with a poor prognosis in breast cancer [82]. Interestingly, when analyzing survival patterns by receptor expression subtypes, co-expression of CD47 and GRP78 negatively correlated with survival in ER+ but not in ER− breast cancer cases, suggesting a possible estrogen effect. Antiestrogen resistant LCC9 breast cancer cells expressed higher cell surface CD47 levels, when compared with the parental antiestrogen therapy sensitive LCC1 cell

line, as determined by flow cytometry [82]. Previous studies showed that LCC9 breast cancer cells express elevated GRP78 protein levels when compared with LCC1 cells [11], thereby confirming co-regulation of GRP78 and CD47 in vitro. Using these cell line pairs, we showed knockdown of GRP78 resulted in a decrease in cell surface CD47 expression in both these cell lines further demonstrating a direct connection between these two proteins [82]. While the mechanisms of these interactions remain unknown, examination of LCC9 orthotopic tumors (where CD47 is highly expressed [15]) shows decreased expression of TSP1. However, targeting GRP78 resulted in an increase of TSP1 expression in vivo [82]. Since TSP1 alone has been shown to regulate macrophage cytolytic activity against cancer cells [83], it is possible that the TSP1/CD47 interaction regulates macrophage infiltration through interplay with GRP78. In a recent study, our group showed that UPR signaling regulated macrophage polarization and responses to immunotherapy [57]. Whether these interactions also involve CD47 is unknown; however, other studies showed that CD47 is involved in macrophage differentiation [84]. Aside from modulating the innate immune response, CD47 has also been implicated in the regulation of adaptive responses against tumors [85]. While currently unknown, it is reasonable to hypothesize that the harsh conditions of the tumor microenvironment may promote immune UPR signaling leading to the dysregulation of both innate and adaptive immune functions regulated in part by CD47 expression and interaction with its ligands.

Conclusion

While originally implicated as an endoplasmic reticulum localized protein, GRP78 can be cytosolic, mitochondrial, located on the plasma membrane, and even secreted from certain cell types. GRP78 is a multifunctional protein that depending on cell stress levels may exist in multiple subcellular compartments impacting a plethora of signaling pathways to control cell fate. Literature indicated GRP78 may control apoptosis, autophagy, metabolism, cellular bioenergetics, immune cell activity, and immunosurveillance. GRP78 may therefore be an attractive target for the treatment of various cancer types to enhance cell death and prevent therapeutic resistance.

References

1. Clarke R, Cook KL, Hu R, Facey CO, Tavassoly I, Schwartz JL, et al. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. Cancer Res. 2012;72:1321–31.
2. Haas IG, Wabl M. Immunoglobulin heavy chain binding protein. Nature. 1983;306:387–9.
3. Pouyssegur J, Shiu RP, Pastan I. Induction of two transformation-sensitive membrane polypeptides in normal fibroblasts by a block in glycoprotein synthesis or glucose deprivation. Cell. 1977;11:941–7.

4. Gething MJ. Role and regulation of the ER chaperone BiP. Semin Cell Dev Biol. 1999;10:465–72.
5. Hendershot LM. The ER function BiP is a master regulator of ER function. Mt Sinai J Med. 2004;71:289–97.
6. Lee AS. The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci. 2001;26:504–10.
7. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature. 2014;508:103–7.
8. Davies MP, Barraclough DL, Stewart C, Joyce KA, Eccles RM, Barraclough R, et al. Expression and splicing of the unfolded protein response gene XBP-1 are significantly associated with clinical outcome of endocrine-treated breast cancer. Int J Cancer. 2008;123:85–8.
9. Gomez BP, Riggins RB, Shajahan AN, Klimach U, Wang A, Crawford AC, et al. Human X-box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. FASEB J. 2007;21:4013–27.
10. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang HB, et al. NF-kappaB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol. 2015;35:379–90.
11. Cook KL, Shajahan AN, Warri A, Jin L, Hilakivi-Clarke LA, Clarke R. Glucose-regulated protein 78 controls cross-talk between apoptosis and autophagy to determine antiestrogen responsiveness. Cancer Res. 2012;72:3337–49.
12. Gazit G, Lu J, Lee AS. De-regulation of GRP stress protein expression in human breast cancer cell lines. Breast Cancer Res Treat. 1999;54:135–46.
13. Scriven P, Coulson S, Haines R, Balasubramanian S, Cross S, Wyld L. Activation and clinical significance of the unfolded protein response in breast cancer. Br J Cancer. 2009;101:1692–8.
14. Fu Y, Li J, Lee AS. GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen starvation-induced apoptosis. Cancer Res. 2007;67:3734–40.
15. Cook KL, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, Warri A, et al. Endoplasmic reticulum stress protein GRP78 modulates lipid metabolism to control drug sensitivity and antitumor immunity in breast cancer. Cancer Res. 2016;76:5657–70.
16. Dong D, Ni M, Li J, Xiong S, Ye W, Virrey JJ, et al. Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. Cancer Res. 2008;68:498–505.
17. Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, et al. A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Res. 2011;71:2848–57.
18. Lee E, Nichols P, Spicer D, Groshen S, Yu MC, Lee AS. GRP78 as a novel predictor of responsiveness to chemotherapy in breast cancer. Cancer Res. 2006;66:7849–53.
19. Xie J, Tao ZH, Zhao J, Li T, Wu ZH, Zhang JF, et al. Glucose regulated protein 78 (GRP78) inhibits apoptosis and attentinutes chemosensitivity of gemcitabine in breast cancer cell via AKT/mitochondrial apoptotic pathway. Biochem Biophys Res Commun. 2016;474:612–9.
20. Lee E, Nichols P, Groshen S, Spicer D, Lee AS. GRP78 as potential predictor for breast cancer response to adjuvant taxane therapy. Int J Cancer. 2011;128:726–31.
21. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem. 2003;278:20915–24.
22. Zhou H, Zhang Y, Fu Y, Chan L, Lee AS. Novel mechanism of anti-apoptotic function of 78-kDa glucose-regulated protein (GRP78): endocrine resistance factor in breast cancer, through release of B-cell lymphoma 2 (BCL-2) from BCL-2-interacting killer (BIK). J Biol Chem. 2011;286:25687–96.
23. Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer. 2014;14:263–76.

24. Wang M, Ye R, Barron E, Baumeister P, Mao C, Luo S, et al. Essential role of the unfolded protein response regulator GRP78/BiP in protection from neuronal apoptosis. Cell Death Differ. 2010;17:488–98.
25. Fu Y, Wey S, Wang M, Ye R, Liao CP, Roy-Burman P, et al. Pten null prostate tumorigenesis and AKT activation are blocked by targeted knockout of ER chaperone GRP78/BiP in prostate epithelium. Proc Natl Acad Sci U S A. 2008;105:19444–9.
26. Wey S, Luo B, Tseng CC, Ni M, Zhou H, Fu Y, et al. Inducible knockout of GRP78/BiP in the hematopoietic system suppresses Pten-null leukemogenesis and AKT oncogenic signaling. Blood. 2012;119:817–25.
27. Cook KL, Shajahan AN, Clarke R. Autophagy and endocrine resistance in breast cancer. Expert Rev Anticancer Ther. 2011;11:1283–94.
28. Cook KL, Warri A, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, et al. Hydroxychloroquine inhibits autophagy to potentiate antiestrogen responsiveness in ER+ breast cancer. Clin Cancer Res. 2014;20:3222–32.
29. Cook KL, Clarke PA, Parmar J, Hu R, Schwartz-Roberts JL, Abu-Asab M, et al. Knockdown of estrogen receptor-alpha induces autophagy and inhibits antiestrogen-mediated unfolded protein response activation, promoting ROS-induced breast cancer cell death. FASEB J. 2014;28:3891–905.
30. Vidal RL, Hetz C. Unspliced XBP1 controls autophagy through FoxO1. Cell Res. 2013;23:463–4.
31. Gade P, Kimball AS, DiNardo AC, Gangwal P, Ross DD, Boswell HS, et al. Death-associated protein Kinase-1 expression and autophagy in chronic lymphocytic leukemia are dependent on activating transcription Factor-6 and CCAAT/enhancer-binding protein-beta. J Biol Chem. 2016;291:22030–42.
32. Li J, Ni M, Lee B, Barron E, Hinton DR, Lee AS. The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. Cell Death Differ. 2008;15:1460–71.
33. Cook KL, Clarke R. Heat shock 70 kDa protein 5/glucose-regulated protein 78 “AMP”ing up autophagy. Autophagy. 2012;8:1827–9.
34. Zhang XY, Zhang TT, Song DD, Zhou J, Han R, Qin ZH, et al. Endoplasmic reticulum chaperone GRP78 is involved in autophagy activation induced by ischemic preconditioning in neural cells. Mol Brain. 2015;8:20.
35. Abdel Malek MA, Jagannathan S, Malek E, Sayed DM, Elgammal SA, Abd El-Azeem HG, et al. Molecular chaperone GRP78 enhances aggresome delivery to autophagosomes to promote drug resistance in multiple myeloma. Oncotarget. 2015;6:3098–110.
36. Zhang Y, Liu R, Ni M, Gill P, Lee AS. Cell surface relocalization of the endoplasmic reticulum chaperone and unfolded protein response regulator GRP78/BiP. J Biol Chem. 2010;285:15065–75.
37. Shin BK, Wang H, Yim AM, Le NF, Brichory F, Jang JH, et al. Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. J Biol Chem. 2003;278:7607–16.
38. Tsai YL, Zhang Y, Tseng CC, Stanciauskas R, Pinaud F, Lee AS. Characterization and mechanism of stress-induced translocation of 78-kilodalton glucose-regulated protein (GRP78) to the cell surface. J Biol Chem. 2015;290:8049–64.
39. Zhang Y, Tseng CC, Tsai YL, Fu X, Schiff R, Lee AS. Cancer cells resistant to therapy promote cell surface relocalization of GRP78 which complexes with PI3K and enhances PI(3,4,5)P3 production. PLoS One. 2013;8:e80071.
40. Yao X, Liu H, Zhang X, Zhang L, Li X, Wang C, et al. Cell surface GRP78 accelerated breast cancer cell proliferation and migration by activating STAT3. PLoS One. 2015;10:e0125634.
41. Misra UK, Gonzalez-Gronow M, Gawdi G, Hart JP, Johnson CE, Pizzo SV. The role of Grp 78 in alpha 2-macroglobulin-induced signal transduction. Evidence from RNA interference that the low density lipoprotein receptor-related protein is associated with, but not necessary for, GRP 78-mediated signal transduction. J Biol Chem. 2002;277:42082–7.

42. Shani G, Fischer WH, Justice NJ, Kelber JA, Vale W, Gray PC. GRP78 and Cripto form a complex at the cell surface and collaborate to inhibit transforming growth factor beta signaling and enhance cell growth. Mol Cell Biol. 2008;28:666–77.
43. Zoni E, Chen L, Karkampouna S, Granchi Z, Verhoef EI, La MF, et al. CRIPTO and its signaling partner GRP78 drive the metastatic phenotype in human osteotropic prostate cancer. Oncogene. 2017;36:4739–49.
44. Raiter A, Yerushalmi R, Hardy B. Pharmacological induction of cell surface GRP78 contributes to apoptosis in triple negative breast cancer cells. Oncotarget. 2014;5:11452–63.
45. Davidson DJ, Haskell C, Majest S, Kherzai A, Egan DA, Walter KA, et al. Kringle 5 of human plasminogen induces apoptosis of endothelial and tumor cells through surface-expressed glucose-regulated protein 78. Cancer Res. 2005;65:4663–72.
46. McFarland BC, Stewart J Jr, Hamza A, Nordal R, Davidson DJ, Henkin J, et al. Plasminogen kringle 5 induces apoptosis of brain microvessel endothelial cells: sensitization by radiation and requirement for GRP78 and LRP1. Cancer Res. 2009;69:5537–45.
47. Yerushalmi R, Raiter A, Nalbandyan K, Hardy B. Cell surface GRP78: a potential marker of good prognosis and response to chemotherapy in breast cancer. Oncol Lett. 2015;10:2149–55.
48. Fu R, Yang P, Wu HL, Li ZW, Li ZY. GRP78 secreted by colon cancer cells facilitates cell proliferation via PI3K/Akt signaling. Asian Pac J Cancer Prev. 2014;15:7245–9.
49. Gonzalez-Gronow M, Cuchacovich M, Llanos C, Urzua C, Gawdi G, Pizzo SV. Prostate cancer cell proliferation in vitro is modulated by antibodies against glucose-regulated protein 78 isolated from patient serum. Cancer Res. 2006;66:11424–31.
50. Li R, Yanjiao G, Wubin H, Yue W, Jianhua H, Huachuan Z, et al. Secreted GRP78 activates EGFR-SRC-STAT3 signaling and confers the resistance to sorafenib in HCC cells. Oncotarget. 2017;8:19354–64.
51. Tsunemi S, Nakanishi T, Fujita Y, Bouras G, Miyamoto Y, Miyamoto A, et al. Proteomics-based identification of a tumor-associated antigen and its corresponding autoantibody in gastric cancer. Oncol Rep. 2010;23:949–56.
52. Politou M, Naresh K, Terpos E, Crawley D, Lampert I, Apperley JF, et al. Anti-angiogenic effect of bortezomib in patients with multiple myeloma. Acta Haematol. 2005;114:170–3.
53. Peng Y, Li Z, Li Z. GRP78 secreted by tumor cells stimulates differentiation of bone marrow mesenchymal stem cells to cancer-associated fibroblasts. Biochem Biophys Res Commun. 2013;440:558–63.
54. Sun FC, Wei S, Li CW, Chang YS, Chao CC, Lai YK. Localization of GRP78 to mitochondria under the unfolded protein response. Biochem J. 2006;396:31–9.
55. Li N, Zoubeidi A, Beraldi E, Gleave ME. GRP78 regulates clusterin stability, retrotranslocation and mitochondrial localization under ER stress in prostate cancer. Oncogene. 2013;32:1933–42.
56. Teng Y, Ren X, Li H, Shull A, Kim J, Cowell JK. Mitochondrial ATAD3A combines with GRP78 to regulate the WASF3 metastasis-promoting protein. Oncogene. 2016;35:333–43.
57. Soto-Pantoja DR, Wilson AS, Clear KYJ, Westwood B, Triozzi PL, Cook KL. Unfolded protein response signaling impacts macrophage polarity to modulate breast cancer cell clearance and melanoma immune checkpoint therapy responsiveness. Oncotarget. 2017;8(46):80545–59.
58. Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. J Clin Invest. 2009;119:1201–15.
59. Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, et al. Structural and functional features and significance of the physical linkage between ER and mitochondria. J Cell Biol. 2006;174:915–21.
60. Prasad M, Pawlak KJ, Burak WE, Perry EE, Marshall B, Whittal RM, et al. Mitochondrial metabolic regulation by GRP78. Sci Adv. 2017;3:e1602038.
61. Bose HS, Sugawara T, Strauss JF III, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med. 1996;335:1870–8.

62. Jiralerspong S, Kim ES, Dong W, Feng L, Hortobagyi GN, Giordano SH. Obesity, diabetes, and survival outcomes in a large cohort of early-stage breast cancer patients. Ann Oncol. 2013;24:2506–14.
63. Petrelli JM, Calle EE, Rodriguez C, Thun MJ. Body mass index, height, and postmenopausal breast cancer mortality in a prospective cohort of US women. Cancer Causes Control. 2002;13:325–32.
64. Rock CL, Demark-Wahnefried W. Nutrition and survival after the diagnosis of breast cancer: a review of the evidence. J Clin Oncol. 2002;20:3302–16.
65. Fontanella C, Lederer B, Gade S, Vanoppen M, Blohmer JU, Costa SD, et al. Impact of body mass index on neoadjuvant treatment outcome: a pooled analysis of eight prospective neoadjuvant breast cancer trials. Breast Cancer Res Treat. 2015;150:127–39.
66. Pierobon M, Frankenfeld CL. Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat. 2013;137:307–14.
67. Widschwendter P, Friedl TW, Schwentner L, DeGregorio N, Jaeger B, Schramm A, et al. The influence of obesity on survival in early, high-risk breast cancer: results from the randomized SUCCESS A trial. Breast Cancer Res. 2015;17:129.
68. Goodwin PJ. Obesity and endocrine therapy: host factors and breast cancer outcome. Breast. 2013;22(Suppl 2):S44–7.
69. Gutierrez MC, Detre S, Johnston S, Mohsin SK, Shou J, Allred DC, et al. Molecular changes in tamoxifen-resistant breast cancer: relationship between estrogen receptor, HER-2, and p38 mitogen-activated protein kinase. J Clin Oncol. 2005;23:2469–76.
70. Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. Nat Rev Cancer. 2009;9:631–43.
71. de AE, McCaskill-Stevens W, Francis P, Quinaux E, Crown JP, Vicente M, et al. The effect of body mass index on overall and disease-free survival in node-positive breast cancer patients treated with docetaxel and doxorubicin-containing adjuvant chemotherapy: the experience of the BIG 02-98 trial. Breast Cancer Res Treat. 2010;119:145–53.
72. Litton JK, Gonzalez-Angulo AM, Warneke CL, Buzdar AU, Kau SW, Bondy M, et al. Relationship between obesity and pathologic response to neoadjuvant chemotherapy among women with operable breast cancer. J Clin Oncol. 2008;26:4072–7.
73. Iyengar NM, Hudis CA, Dannenberg AJ. Obesity and inflammation: new insights into breast cancer development and progression. Am Soc Clin Oncol Educ Book. 2013;46–51.
74. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. Nat Rev Cancer. 2011;11:886–95.
75. Sumis A, Cook K, Andrade FO, Hu R, Kidney E, Zhang X, et al. Social isolation induces autophagy in the mouse mammary gland: link to increased mammary cancer risk. Endocr Relat Cancer. 2016;23(10):839–56.
76. Fang L, Kojima K, Zhou L, Crossman DK, Mobley JA, Grams J. Analysis of the human proteome in subcutaneous and visceral fat depots in diabetic and non-diabetic patients with morbid obesity. J Proteomics Bioinform. 2015;8:133–41.
77. Matsuo K, Gray MJ, Yang DY, Srivastava SA, Tripathi PB, Sonoda LA, et al. The endoplasmic reticulum stress marker, glucose-regulated protein-78 (GRP78) in visceral adipocytes predicts endometrial cancer progression and patient survival. Gynecol Oncol. 2013;128:552–9.
78. Banuls C, Rovira-Llopis S, Lopez-Domenech S, Diaz-Morales N, Blas-Garcia A, Veses S, et al. Oxidative and endoplasmic reticulum stress is impaired in leukocytes from metabolically unhealthy vs healthy obese individuals. Int J Obes. 2017;41(10):1556–63.
79. Soto-Pantoja DR, Stein EV, Rogers NM, Sharifi-Sanjani M, Isenberg JS, Roberts DD. Therapeutic opportunities for targeting the ubiquitous cell surface receptor CD47. Expert Opin Ther Targets. 2013;17:89–103.
80. Soto-Pantoja DR, Kaur S, Roberts DD. CD47 signaling pathways controlling cellular differentiation and responses to stress. Crit Rev Biochem Mol Biol. 2015;50:212–30.

81. Chao MP, Jaiswal S, Weissman-Tsukamoto R, Alizadeh AA, Gentles AJ, Volkmer J, et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. Sci Transl Med. 2010;2:63ra94.
82. Cook KL, Soto-Pantoja DR. "UPRegulation" of CD47 by the endoplasmic reticulum stress pathway controls anti-tumor immune responses. Biomark Res. 2017;5:26.
83. Martin-Manso G, Galli S, Ridnour LA, Tsokos M, Wink DA, Roberts DD. Thrombospondin 1 promotes tumor macrophage recruitment and enhances tumor cell cytotoxicity of differentiated U937 cells. Cancer Res. 2008;68:7090–9.
84. Sakakura K, Takahashi H, Kaira K, Toyoda M, Murata T, Ohnishi H, et al. Relationship between tumor-associated macrophage subsets and CD47 expression in squamous cell carcinoma of the head and neck in the tumor microenvironment. Lab Investig. 2016;96:994–1003.
85. Soto-Pantoja DR, Terabe M, Ghosh A, Ridnour LA, DeGraff WG, Wink DA, et al. CD47 in the tumor microenvironment limits cooperation between antitumor T-cell immunity and radiotherapy. Cancer Res. 2014;74:6771–83.

Chapter 10
Autophagy in Neurodegenerative Diseases

Charbel Moussa

Abstract It is estimated that the prevalence of neurodegenerative diseases will overtake cancer incidence to become the second most common cause of death after cardiovascular disease. Many of these diseases are associated with the inappropriate accumulation of proteins that are toxic to neurons. Normally, the accumulation of these proteins would be prevented by the appropriate activation of autophagy, a key cellular function regulated by activation of an endoplasmic reticulum stress and the unfolded protein response. This chapter discusses the role of autophagy in neurodegenerative disease, with a detailed description of how autophagy is activated and executed.

Keywords Alzheimer's disease · Apoptosis · Autophagic vacuole · Autophagosome · Beclin1 · Chaperone-mediated · CNS · Endosome · Lewy Body Dementia · Lysosome · Mitochondria · Neuron · Parkin · Parkinson's disease · PINK1 · Post-mitotic · Tauopathy · Tyrosine kinase inhibitor

Introduction

Autophagy is a normal cellular quality control mechanism involved in recycling materials and maintaining homeostasis. Activation of autophagy can promote either death or survival of cells. Changes in autophagy are seen in most neurodegenerative diseases. Dysfunction of autophagy in neurodegeneration is predominantly reflected as an accumulation of cytosolic vacuoles that are either too immature to be transported or unable to fuse with the lysosome for recycling. Neuronal death may be caused by arrest of autophagic flux, due to: disruption of clearance mechanisms of key neurotoxic proteins as a result of lack of maturation of vacuoles that sequester misfolded proteins; or inefficient fusion of autophagic vacuoles with the lysosome;

C. Moussa (✉)
Laboratory for Dementia and Parkinsonism, Translational Neurotherapeutics Program, Department of Neurology, Georgetown University Medical Center, Washington, DC, USA
e-mail: cem46@georgetown.edu

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2_10

or loss of the degradative enzyme functions within the lysosome. Completion of autophagy through induction, maturation of vacuoles and fusion with the lysosome is thus indispensable for clearance of cellular debris. This chapter discusses the role of autophagy in neuronal survival and the impact of its failure on neurodegenerative diseases.

Autophagy in Post-mitotic Neurons

Autophagy is a normal physiological process that includes formation of autophagic vacuoles and delivery to lysosomes for proteolytic degradation. Normal autophagy is a dynamic multi-step process that includes several compartments. Autophagy is generally initiated to form the sequestering phagophore [1], which develops into a double-membrane vacuole called the autophagosome [2]. Autophagosomes can mature to generate the amphisome via fusion with endosomes [3, 4], and the autolysosome/autophagolysosome via fusion (of autophagosomes or amphisomes) with a lysosome [2]. Autophagy is a common quality control mechanism shared by mitotic and post-mitotic cells and it is frequently activated in cancer to accelerate clearance of unwanted oncogenes [5]. Drugs that can induce autophagy may halt mitotic cell division and tumor growth in cancer. Autophagy can degrade oncogenes, tumor suppressor genes, damaged cytosolic components and organelles like the endoplasmic reticulum (ER), mitochondria and DNA to control mitotic division. Autophagy can also lead to self-destruction of cancer cells via promotion of apoptosis and activation of the tumor suppressor p53 in response to DNA and organelle damage (e.g., ER, Golgi) to arrest proliferation and promote survival. However, if DNA damage cannot be resolved, p53 triggers apoptotic death [6, 7]. Triggering various signaling mechanisms that mediate signaling via the late endosomal-lysosomal pathway increases autophagic degradation [8–10] and limits tumor growth. Malignant tumors spread into nearby tissues by cellular contiguity or metastasize via blood and/or lymphatic transport. The spread of toxic or misfolded proteins in neurodegeneration may be similar to the spread of metastatic cancer, as both pathologies spread from the place where they originate and lodge in nearby tissues. In mitotic cancer cells, cell division and apoptosis are mediated by signaling mechanisms via the endosomal (early and recycling) system [8]. Similarly, the spread of un-degraded neurotoxic proteins that can be secreted from dying neurons may rely on cellular contiguity and propagation of toxic or prion-like proteins, analogous to oncogenes, along neuroanatomical pathways [11], leading to progressive spread of disease and cell death.

Neurons are post-mitotic brain cells and their survival determines our entire mental, cognitive, motor, and other central nervous system (CNS) functions. Neurodegenerative diseases include a group of genetic and sporadic disorders that result from neuronal death and progressive CNS dysfunction. The CNS does not allow tissue regeneration so when post-mitotic neurons die, there is no replacement.

In neurodegeneration, failure of cellular quality control mechanisms leads to inefficient protein degradation via the proteasome or autophagy [12], resulting in intracellular accumulation of misfolded and toxic proteins. Consequently, neurotoxic proteins in CNS may be secreted from a pre-synaptic neuron across the synapse to invade post-synaptic neurons. Under normal conditions, excess proteins are recycled via the endosomal-lysosomal system to prevent protein accumulation and/or secretion [8–10].

Protein Accumulation in Neurodegenerative Diseases

Accumulation of toxic proteins including α-synuclein (Lewy bodies), beta-amyloid plaques, tau tangles, huntingtin, prions, and transactivation DNA/RNA binding protein (TDP)-43 are major culprits in neurodegeneration. These toxic proteins trigger progressive neurodegeneration leading to loss of CNS functions, including central and autonomic control. There is compelling evidence that toxic protein propagation from cell to cell [11] leads to onset and progression of neurodegeneration. Defects in autophagy are well recognized in neurodegenerative diseases [13–19] and are characterized by un-degraded or increased number of autophagic vacuoles [14, 15, 20]. Neuronal accumulation of autophagosomes has been described in multiple brain disorders [21–23], including advanced stages [24] and animal models [25] of AD, suggesting reduced lysosomal clearance [26]. Dysfunction in autophagy is seen in several other neurodegenerative diseases [15, 27–29] including Parkinson's disease (PD) and Lewy Body Dementia (LBD). α-synuclein accumulation in Lewy Bodies (LBs) may be caused by inadequate protein clearance via chaperone-mediated autophagy and reduced lysosomal function [30–32].

Impairment of the autophagic pathway could come from dysfunction in any of several steps of autophagy. Firstly, induction of autophagy can become detrimental when un-degraded autophagosomes accumulate and overwhelm the lysosome (Fig. 10.1). Inhibition of the mammalian target of rapamycin (mTOR) leads to induction of autophagy [33–35] and increases β-amyloid (Aβ) degradation by the lysosomal system [36]. mTOR inhibition attenuates Aβ levels in triple transgenic AD mice [37], suggesting mTOR involvement in autophagic induction. Conversely, accumulation of intraneuronal Aβ also increases mTOR activity [37] and accumulation of autophagic vacuoles [38, 39]. Secondly, inefficient recognition of aggregate proteins or defective organelles has also been described in models of neurodegeneration [40], whereby molecular steps of autophagy are activated, but autophagosome clearance due to inefficient fusion with the lysosome is defective [41]. In patients with AD, the levels of a key autophagy protein complex known as beclin-1 are decreased [42], suggesting inefficient execution of beclin-dependent autophagy and increased Aβ and hyper-phosphorylated tau (p-tau) accumulation. Lentiviral delivery to express beclin-1 activates autophagy and improves neurodegenerative pathology in α-synuclein mouse models of PD [43] and AD [42]. Thirdly, the final

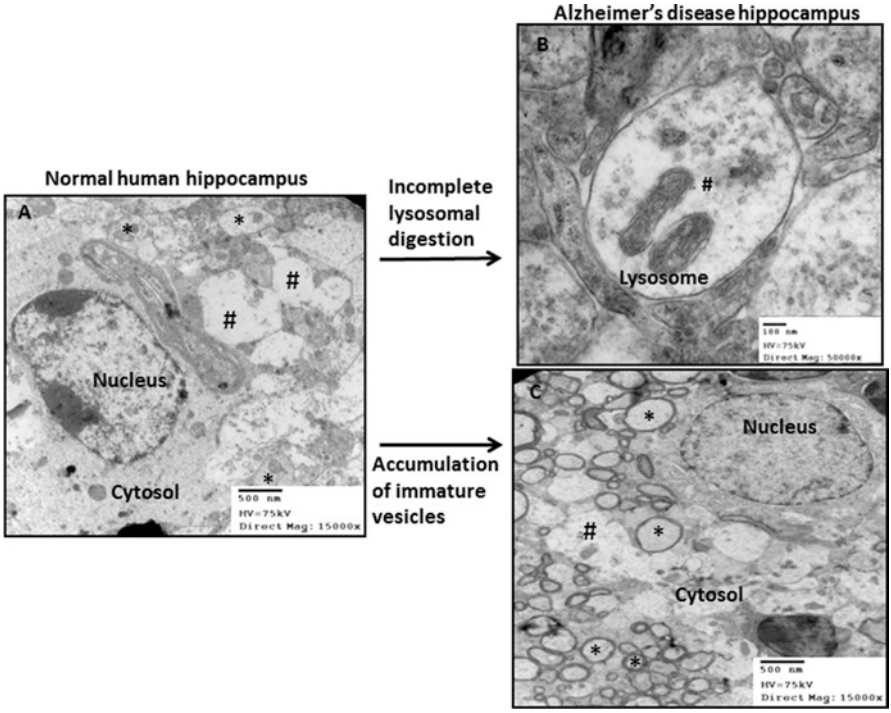

Fig. 10.1 (**a**) Electron micrographs of hippocampus from a normal human subject showing nucleus and cytosolic vacuoles. Asterisks (*) indicate pre-lysosomal vesicles (autophagosomes/amphisomes) and (#) indicate fusing vesicles to form autophagolysosomes/autolysosomes under normal conditions. Electron micrograph of hippocampus from aged-matched Alzheimer's patient showing (**b**) undigested autophagosomes in lysosomes (#) that accumulate debris and cell organelles and (**c**) accumulation (*) of immature cytosolic vesicles indicative of either lack of fusion with the lysosomes (#) or overwhelming lysosomal clearance

step of autophagy following induction and formation/maturation of autophagosomes is lysosomal degradation. Aβ accumulates in the brain of AD mouse models and leads to defective proteolytic clearance, while enhancement of proteolytic degradation via the lysosome increases the rate of autophagic protein turnover [44].

Fusion of Autophagic Vacuoles

It appears that tau malfunction is a common mechanism that underlies autophagic dysfunctions in neurodegeneration. In the adult human brain, tau exists as six protein isoforms that differ by the presence of either 0, 1, or 2 N-terminal inserts and either three (3R) or four (4R) microtubule binding repeats located at the C terminus of the protein [45, 46]. Microtubule Associated Protein Tau (MAPT) gene expresses tau, which binds to and stabilizes microtubules via its C-terminal repeats in a

process regulated by phosphorylation [47]. More evidence is emerging about the relationship between tau modification and alteration of normal autophagy. Functional autophagy is negatively regulated by the serine/threonine protein kinase mTOR [48], which can be inhibited to activate autophagy and slow the progress of neurodegeneration [28]. Different mechanisms can lead to formation of autophagosomes, including mTOR-dependent and mTOR-independent autophagy [49–51], and beclin-independent autophagy [52].

Autophagosomes recruit lysosomes via retrograde transport on microtubules, requiring an intact microtubule cytoskeleton and cytoplasmic histone deacetylase (HDAC6) to mediate the fusion of autophagosome with the lysosome [53]. Tau regulates HDAC6 function, resulting in decreased degradation of aggregated proteins [54], suggesting that HDAC6 is a tau-interacting protein and potential modulator of tau phosphorylation and accumulation [55]. Tau phosphorylation causes stabilization of microtubules [47]. Reduction of tau expression in cellular models of Niemann–Pick type C disease is associated with decreased induction and degradation of autophagic vacuoles [56, 57], supporting the notion that normal autophagy is dependent on trafficking along microtubules [53] and its impairment can cause neurodegeneration [58, 59]. Hyper-phosphorylation of tau leads to its dissociation from microtubules and aggregation [60], leading to alteration of tau protein function and impaired neuronal transport [60].

Inhibition of autophagy enhances tau aggregation and cytotoxicity, and lysosomal function contributes to degradation of tau [61–63]. Lentiviral expression of 4R wild type tau or its mutants results in differential hyper-phosphorylation and aggregation [64]. The most direct evidence of the dependence of autophagic clearance on intact tau-associated microtubules is introduction of Aβ into $Tau^{-/-}$ mice that results into secretion of intraneuronal Aβ, while co-expression of tau and Aβ together in the same mice results in less Aβ secretion and degradation of both tau and Aβ via the lysosome [65]. Tau-mediated stabilization of microtubules may facilitate organelles movement. Aggregation of mitochondria at the distal ends of axons in $tau^{-/-}$ primary neuronal culture suggests that movement of organelles is altered in these mice [66].

Tauopathies are pathologically characterized by accumulation of hyperphosphorylated, insoluble aggregates of tau in neurons and glia of affected brain regions. In AD, neurofibrillary tangles (NFTs) comprised of insoluble p-tau are characteristic features, alongside extracellular plaques composed of Aβ peptide [67]. The increase of Aβ production, due to AD mutations, led to the development of the amyloid cascade hypothesis, where tau pathology is considered downstream of Aβ in the pathological cascade [68, 69]. The presence of NFTs correlates highly with the degree of dementia in AD suggesting a central role for tau in neuronal death [70]. Disruption in tau splicing is observed in both familial Fronto-Temporal Lobar Degeneration (FTLD) and Progressive Supranuclear Palsy (PSP), Corticobasal Degeneration (CBD), and Fronto-Temporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17) [71, 72]. These conditions are clinically manifest with dementia and Parkinsonism, and pathologically characterized by tau deposition [73–76].

There are two historical examples of tau involvement in epidemic Parkinsonism: post-encephalitic Parkinsonism and the Parkinsonism/dementia complex (PDC) of Guam [77, 78]. Increased 4R tau is also detected in PD brain, mainly striatum, suggesting that common mechanisms of increased expression underlie the association of tau gene (MAPT) with PD, PSP, and CBD [79]. Recent genome wide association studies (GWAS) identified MAPT as a genetic risk factor for PD [80]. However, inconsistent tau pathology in idiopathic PD makes the genetic link puzzling, especially as MAPT has not been identified as a risk factor in other GWAS of PD [81] or AD, where NFT pathology is extensive [82].

Why should variation in tau be a risk factor for a disease where tau pathology is not consistently observed, but not influence disease risk where tau deposition is prevalent? The effects of tau on pathological mechanisms in neurodegeneration may be a common denominator due to its role in neuronal transport and execution of autophagy. Some studies suggest that a loss of tau leads to neurodegeneration [83, 84], cognitive dysfunction, and neuropathology similar to tauopathy in FTLD-17 [83–85]. Other reports show that reduction of endogenous levels of tau abrogate amyloid-induced neuronal toxicity [86]. The effects of tau may be downstream in neurodegenerative pathologies, since transgenic animals over-expressing malfunctioning tau (p-tau) or Tau−/− mice develop autophagic defects in response to α-synuclein or Aβ accumulation**.** $Tau^{-/-}$ mice provide an important insight into the role of tau as a neuronal microtubule associated protein involved in stabilization of axonal microtubules [87, 88], neuronal maturation [88], axonal transport [66], and long-term potentiation [89].

Variation at MAPT influences PD risk where tangles are not found consistently, but has only a moderate influence on AD risk, where NFTs are a defining pathological feature [90]. Thus, amyloid accumulation may be responsible for neurodegeneration due to disruption of tau function that facilitates autophagy via axonal transport. Tau modification and cell death in lentiviral gene transfer animal models occur as a result of expression of intracellular Aβ [91] or α-synuclein [92], suggesting that tau modification is triggered by amyloid proteins that can destabilize microtubules that provide the "railway" for autophagosomal transport to fuse with the lysosomes. While duplications and triplications of α-synuclein cause PD, and AD-type pathology exists in Down's syndrome with triplication of the amyloid precursor protein (APP) gene [93], tau levels are high in tauopathy, AD, and PD post-mortem tissue [94]. Thus, increased levels of non-mutated tau can lead to p-tau and cause neurodegeneration. In FTLD, tau oligomers are toxic [95]. In AD, tau pathology correlates strongly with dementia and the extent of neuronal loss [96]. Furthermore, several studies show that tau is necessary for Aβ-mediated toxicity; for example, primary neuronal cultures from $Tau^{-/-}$ mice are resistant to Aβ, and crossing APP transgenic with $Tau^{-/-}$ mice reduces Aβ induced deficits [97, 98]. These findings suggest that tau may be required to mediate the toxic events leading to protein accumulation or clearance of toxic proteins in neurodegeneration.

Maturation of Autophagic Vacuoles

Normal autophagy involves stepwise maturation of autophagosomes and fusion with both endosomal and lysosomal vesicles. Several molecules can regulate the maturation of autophagosomes, including the AAA ATPase SKD1, the small GTP binding protein Rab7, and the AD-linked presenilin 1 [99]. Autophagosome accumulation could be due to lack of maturation, leading to inefficient fusion with lysosomes. Ubiquitination may facilitate recognition between components of the autophagic machinery [50, 100] and may promote autophagosome maturation [101, 102]. The molecular steps of autophagy can be activated, but autophagosome clearance remains insufficient, due to inefficient fusion with the lysosome [41]. Autophagy like proteins, called Atgs, are critical in determining autophagic vacuole formation [53]. Atg5, Atg7, and Atg12 determine the sequestering phagophore formation [103–108]. Light Chain (LC)-3 protein is initially synthesized in an unprocessed form, proLC3, but sequential ubiquitination-like reaction and conjugation of Atgs lead to its modification into LC3-II, which is a marker of mature autophagosomes [109, 110]. LC3-II can also be localized to phagophores, the precursor of autophagosomes. Reduced turnover of autophagosomes can be due to inhibition of their maturation, leading to inability to fuse with the lysosome [111], which is evident in neurodegeneration (Fig. 10.1).

Parkin is an E3 ubiquitin ligase that facilitates proteasomal degradation of misfolded proteins [112]. Loss of function mutation, i.e., threonine to arginine (T240R) in the gene coding for the E3-ubiquitin ligase parkin, is associated with juvenile onset autosomal recessive PD [113, 114]. PD with parkin mutations, as well as some familial Fronto-Temporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17), often exhibits tau pathology, mainly in the striatum and basal ganglia [115–118]. Parkin is inactivated in the nigrostriatal regions of sporadic PD [119, 120] and decreased parkin levels are detected in AD brains [121]. In PD with parkin mutations, neuronal loss in the substantia nigra (SN) is found with LBs and tau positive astrocytes [122] or NFTs and argyrophilic astrocytes [116, 123]. A Dutch family of PD with parkin mutation shows tau pathology, even in the absence of LBs and NFTs [115]. Different parkin mutations show tau pathology [124, 125], and a PSP patient who is a carrier of a single heterozygous mutation (C212Y) of the parkin gene displays p-tau [124, 125]. In animal models lacking parkin, p-tau aggregates in the cortex and hippocampus [126]. Parkin over-expression attenuates Aβ and α-synuclein toxicity in human M17 neuroblastoma cells over-expressing 4R wild type, but not P301L mutant tau [127]. Activation of autophagy improves dopaminergic cell survival in parkin deficient and tau over-expressing mice [128]. Parkin and PTEN-induced putative kinase 1 (PINK1) regulate mitophagy [129–132] and enhance autophagy in vitro [133]. Parkin ubiquitinates proteins of defective mitochondria and induces LC3 and forms autophagosomes [100, 134–137]. Parkin ubiquitin ligase activity modulates beclin-LC3 mediated autophagy [138], consistent with the role of ubiquiti-

nation in autophagic clearance mechanisms [100, 139]. Parkin activates beclin-mediated autophagy in triple transgenic AD mice [92]. These findings indicate that parkin activation leads to autophagosome maturation and restoration of normal autophagy in neurodegenerative diseases.

The effects of neurotoxic proteins on normal autophagy and vice versa may not be straightforward. Expression of intracellular Aβ or α-synuclein leads to p-tau [91]. Additionally, α-synuclein or Aβ expression stimulates formation of autophagic vacuoles similar to p-tau alone [64]. However, in some circumstances where microtubules are not completely dysfunctional, tau aggregates are removed by autophagy [140, 141]. Cytosolic accumulation of autophagic vacuoles in lentiviral Aβ gene transfer models is similar to triple transgenic AD mice, which derive Aβ from APP and express the human mutant P301L tau [38]. This accumulation of vacuoles is likely due to reduced autophagic flux [142]. Some studies suggest that the phagophore sequestering membrane originates from stressed ER [143], which is required for autophagosome formation [144, 145]. Formation of autophagic vacuoles in animals expressing Aβ with a signal peptide directed to the ER [91] shows impaired autophagy via lack of maturation of full double-membrane autophagosomes and ER fragmentation [38, 146–150]. However, parkin expression or activation via tyrosine kinase inhibitors leads to autophagosome maturation and clearance via the lysosomes [38, 146–150]. These findings suggest that autophagosome maturation is indispensable for protein clearance in neurodegeneration.

There is some evidence to support the early steps of induction via deletion of autophagic components, including Atg7, that suppresses autophagy [58, 59]. LC3 is believed to link ubiquitinated protein aggregates to the autophagosome for degradation [101, 102]. Furthermore, beclin-1 activation is crucial to autophagic flux. Lower beclin levels are reported in brains from older individuals and patients with neurodegenerative diseases [151], whereas an increase in beclin-1 protein levels is associated with autophagic activity [42, 152]. Parkin activation facilitates autophagic degradation of intracellular protein inclusions in animal models in vivo. Loss of parkin activity impairs its interaction with beclin-1, thus reducing amyloid protein clearance in models of neurodegeneration [38, 147, 148, 153, 154]. Functional parkin-beclin-1 interaction is reduced in neurodegeneration, suggesting that aging leads to parkin inactivation independent of disease causing mutations [147, 150, 155]. Tyrosine kinase inhibitors (TKIs) are effective and well-tolerated treatments for chronic myelogenous leukemia (CML) [156, 157]. TKIs nilotinib (BCR-ABL) and bosutinib (SRC-ABL) penetrate the brain and activate parkin [119, 147, 148], leading to interaction with beclin-1 and autophagic protein clearance [38, 147, 148, 153–155]. Therefore, parkin effects on autophagosome maturation may be used as a therapeutic approach in neurodegenerative diseases.

Mitophagy in Neurodegeneration

Parkin activity is regulated by associated proteins, post-translational modification, and self-regulation through intramolecular interactions [158]. Parkin modifies mitochondrial outer membrane proteins and promotes removal of dysfunctional mitochondria [159]. In response to mitochondrial depolarization, PINK1 phosphorylates Serine 65 in the Ubl domain of parkin [160], leading to activation. Recent structural analysis of parkin in an auto-inhibited state provided further insights into how phosphorylation and ubiquitination activate parkin [161, 162]. PINK1 modifies ubiquitin at Serine 65, which is homologous to the site phosphorylated in parkin Ubl domain [163–166]. Phosphorylation of both ubiquitin and parkin are necessary to overcome parkin auto-inhibition [166, 167], allowing parkin self-ubiquitination and recruitment of substrates. Auto-ubiquitination activates parkin to subsequently recruit TDP-43 for ubiquitination [168]. The link between parkin ubiquitination and its E3 ubiquitin ligase activity was demonstrated by several studies showing that regulation of parkin ubiquitination affects its activity and stability [153, 154, 163–165]. Taken together, these findings provide evidence that parkin is a quality control protein that monitors physiological perturbations and regulates autophagy in neurodegeneration and beyond.

Conclusion

Autophagic defects are well recognized as an underlying common mechanism in many neurodegenerative diseases. These defects are predominantly linked with protein accumulation that impairs the steps of normal autophagy, including maturation and fusion of autophagic vacuoles in neurons; or result from an overwhelmed lysosome that can no longer cope with increased protein production and misfolding.

References

1. Seglen PO. Regulation of autophagic protein degradation in isolated liver cells. In: Glaumann H, Ballard FJ, editors. Lysosomes: their role in protein breakdown. London: Academic; 1987. p. 369–414.
2. de Duve C, Wattiaux R. Functions of lysosomes. Annu Rev Physiol. 1966;28:435–92.
3. Dunn WA Jr. Autophagy and related mechanisms of lysosome-mediated protein degradation. Trends Cell Biol. 1994;4(4):139–43.
4. Gordon PB, Seglen PO. Prelysosomal convergence of autophagic and endocytic pathways. Biochem Biophys Res Commun. 1988;151(1):40–7.
5. Macintosh RL, Ryan KM. Autophagy in tumour cell death. Semin Cancer Biol. 2013;23(5):344–51.
6. Crighton D, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell. 2006;126(1):121–34.

7. Yee KS, et al. PUMA- and Bax-induced autophagy contributes to apoptosis. Cell Death Differ. 2009;16(8):1135–45.
8. Mellman I. Endocytosis and molecular sorting. Annu Rev Cell Dev Biol. 1996;12:575–625.
9. Jerram AH, Smith PF, Darlington CL. A dose-response analysis of the behavioral effects of (+)MK-801 in guinea pig: comparison with CPP. Pharmacol Biochem Behav. 1996;53(4):799–807.
10. Luzio JP, et al. Lysosome-endosome fusion and lysosome biogenesis. J Cell Sci. 2000;113(Pt 9):1515–24.
11. Polymenidou M, Cleveland DW. Prion-like spread of protein aggregates in neurodegeneration. J Exp Med. 2012;209(5):889–93.
12. Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp Mol Med. 2015;47:e147.
13. Boland B, et al. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J Neurosci. 2008;28(27):6926–37.
14. Kegel KB, et al. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. J Neurosci. 2000;20(19):7268–78.
15. Nixon RA, et al. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol. 2005;64(2):113–22.
16. Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet. 2002;11(9):1107–17.
17. Sabatini DM. mTOR and cancer: insights into a complex relationship. Nat Rev Cancer. 2006;6(9):729–34.
18. Stefanis L, et al. Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. J Neurosci. 2001;21(24):9549–60.
19. Webb JL, et al. Alpha-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem. 2003;278(27):25009–13.
20. Yang Y, et al. Induction of autophagy in neurite degeneration of mouse superior cervical ganglion neurons. Eur J Neurosci. 2007;26(10):2979–88.
21. Mizushima N, et al. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069–75.
22. Nixon RA, Yang DS, Lee JH. Neurodegenerative lysosomal disorders: a continuum from development to late age. Autophagy. 2008;4(5):590–9.
23. Winslow AR, Rubinsztein DC. Autophagy in neurodegeneration and development. Biochim Biophys Acta. 2008;1782(12):723–9.
24. Lee JH, et al. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell. 2010;141(7):1146–58.
25. Yu WH, et al. Macroautophagy—a novel beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol. 2005;171(1):87–98.
26. Kaasik A, et al. Up-regulation of lysosomal cathepsin L and autophagy during neuronal death induced by reduced serum and potassium. Eur J Neurosci. 2005;22(5):1023–31.
27. Pan T, et al. The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease. Brain. 2008;131(Pt 8):1969–78.
28. Ravikumar B, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet. 2004;36(6):585–95.
29. Sarkar S, et al. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nat Chem Biol. 2007;3(6):331–8.
30. Cuervo AM, et al. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science. 2004;305(5688):1292–5.
31. Martinez-Vicente M, et al. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J Clin Invest. 2008;118(2):777–88.
32. Xilouri M, et al. Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One. 2009;4(5):e5515.

33. Illi B, et al. Nitric oxide modulates chromatin folding in human endothelial cells via protein phosphatase 2A activation and class II histone deacetylases nuclear shuttling. Circ Res. 2008;102(1):51–8.
34. Martin M, et al. Protein phosphatase 2A controls the activity of histone deacetylase 7 during T cell apoptosis and angiogenesis. Proc Natl Acad Sci U S A. 2008;105(12):4727–32.
35. Simboeck E, et al. A phosphorylation switch regulates the transcriptional activation of cell cycle regulator p21 by histone deacetylase inhibitors. J Biol Chem. 2010;285(52):41062–73.
36. Vingtdeux V, et al. Novel synthetic small-molecule activators of AMPK as enhancers of autophagy and amyloid-{beta} peptide degradation. FASEB J. 2011;25(1):219–31.
37. Caccamo A, et al. Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and tau: effects on cognitive impairments. J Biol Chem. 2010;285(17): 13107–20.
38. Khandelwal PJ, et al. Parkin mediates beclin-dependent autophagic clearance of defective mitochondria and ubiquitinated Abeta in AD models. Hum Mol Genet. 2011;20(11):2091–102.
39. Herman AM, Moussa CE. The ubiquitin ligase parkin modulates the execution of autophagy. Autophagy. 2011;7(8):919–21.
40. Wong ES, et al. Autophagy-mediated clearance of aggresomes is not a universal phenomenon. Hum Mol Genet. 2008;17(16):2570–82.
41. He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet. 2009;43:67–93.
42. Pickford F, et al. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. J Clin Invest. 2008;118(6):2190–9.
43. Spencer B, et al. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson's and Lewy body diseases. J Neurosci. 2009;29(43):13578–88.
44. Yang DS, et al. Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. Brain. 2011;134(Pt 1):258–77.
45. Goedert M, et al. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. EMBO J. 1989;8(2):393–9.
46. Andreadis A, Brown WM, Kosik KS. Structure and novel exons of the human tau gene. Biochemistry. 1992;31(43):10626–33.
47. Lindwall G, Cole RD. Phosphorylation affects the ability of tau protein to promote microtubule assembly. J Biol Chem. 1984;259(8):5301–5.
48. Kanazawa T, et al. Amino acids and insulin control autophagic proteolysis through different signaling pathways in relation to mTOR in isolated rat hepatocytes. J Biol Chem. 2004;279(9):8452–9.
49. Sarkar S, et al. A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. Hum Mol Genet. 2008;17(2):170–8.
50. Sarkar S, Ravikumar B, Rubinsztein DC. Autophagic clearance of aggregate-prone proteins associated with neurodegeneration. Methods Enzymol. 2009;453:83–110.
51. Yamamoto A, Cremona ML, Rothman JE. Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. J Cell Biol. 2006;172(5):719–31.
52. Scarlatti F, et al. Role of non-canonical Beclin 1-independent autophagy in cell death induced by resveratrol in human breast cancer cells. Cell Death Differ. 2008;15(8):1318–29.
53. Iwata A, et al. HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin. J Biol Chem. 2005;280(48):40282–92.
54. Perez M, et al. Tau—an inhibitor of deacetylase HDAC6 function. J Neurochem. 2009;109(6):1756–66.
55. Ding H, Dolan PJ, Johnson GV. Histone deacetylase 6 interacts with the microtubule-associated protein tau. J Neurochem. 2008;106(5):2119–30.

56. Pacheco CD, Elrick MJ, Lieberman AP. Tau deletion exacerbates the phenotype of Niemann-Pick type C mice and implicates autophagy in pathogenesis. Hum Mol Genet. 2009;18(5):956–65.
57. Ravikumar B, et al. Dynein mutations impair autophagic clearance of aggregate-prone proteins. Nat Genet. 2005;37(7):771–6.
58. Hara T, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006;441(7095):885–9.
59. Komatsu M, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006;441(7095):880–4.
60. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci. 2007;8(9):663–72.
61. Wang Y, et al. Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. Hum Mol Genet. 2009;18(21):4153–70.
62. Hamano T, et al. Autophagic-lysosomal perturbation enhances tau aggregation in transfectants with induced wild-type tau expression. Eur J Neurosci. 2008;27(5):1119–30.
63. Braak H, et al. Vulnerability of cortical neurons to Alzheimer's and Parkinson's diseases. J Alzheimers Dis. 2006;9(3 Suppl):35–44.
64. Khandelwal PJ, et al. Wild type and P301L mutant tau promote neuro-inflammation and alpha-Synuclein accumulation in lentiviral gene delivery models. Mol Cell Neurosci. 2012;49(1):44–53.
65. Lonskaya I, et al. Tau deletion impairs intracellular beta-amyloid-42 clearance and leads to more extracellular plaque deposition in gene transfer models. Mol Neurodegener. 2014;9:46.
66. Jimenez-Mateos EM, et al. Role of MAP 1B in axonal retrograde transport of mitochondria. Biochem J. 2006;397(1):53–9.
67. Grundke-Iqbal I, et al. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A. 1986;83(13):4913–7.
68. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 1991;12(10):383–8.
69. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256(5054):184–5.
70. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239–59.
71. Bird TD, et al. A clinical pathological comparison of three families with frontotemporal dementia and identical mutations in the tau gene (P301L). Brain. 1999;122(Pt 4):741–56.
72. Gasparini L, Terni B, Spillantini MG. Frontotemporal dementia with tau pathology. Neurodegener Dis. 2007;4(2–3):236–53.
73. Hutton M, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature. 1998;393(6686):702–5.
74. Poorkaj P, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol. 1998;43(6):815–25.
75. Hasegawa M, Smith MJ, Goedert M. Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. FEBS Lett. 1998;437(3):207–10.
76. Jiang Z, et al. Mutations in tau gene exon 10 associated with FTDP-17 alter the activity of an exonic splicing enhancer to interact with Tra2 beta. J Biol Chem. 2003;278(21):18997–9007.
77. Gibbs CJ Jr, Gajdusek DC. Amyotrophic lateral sclerosis, Parkinson's disease, and the amyotrophic lateral sclerosis-parkinsonism-dementia complex on Guam: a review and summary of attempts to demonstrate infection as the aetiology. J Clin Pathol Suppl (R Coll Pathol). 1972;6:132–40.
78. Wong KT, et al. An immunohistochemical study of neurofibrillary tangle formation in post-encephalitic parkinsonism. Clin Neuropathol. 1996;15(1):22–5.
79. Tobin JE, et al. Haplotypes and gene expression implicate the MAPT region for Parkinson disease: the GenePD study. Neurology. 2008;71(1):28–34.

80. Simon-Sanchez J, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet. 2009;41(12):1308–12.
81. Satake W, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet. 2009;41(12):1303–7.
82. Lambert JC, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet. 2009;41(10):1094–9.
83. Dawson HN, et al. Loss of tau elicits axonal degeneration in a mouse model of Alzheimer's disease. Neuroscience. 2010;169(1):516–31.
84. Morris M, et al. The many faces of tau. Neuron. 2011;70(3):410–26.
85. Ikegami S, Harada A, Hirokawa N. Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice. Neurosci Lett. 2000;279(3):129–32.
86. Roberson ED, et al. Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. J Neurosci. 2011;31(2):700–11.
87. Harada A, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. Nature. 1994;369(6480):488–91.
88. Dawson HN, et al. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. J Cell Sci. 2001;114(Pt 6):1179–87.
89. Shipton OA, et al. Tau protein is required for amyloid {beta}-induced impairment of hippocampal long-term potentiation. J Neurosci. 2011;31(5):1688–92.
90. Pittman AM, Fung HC, de Silva R. Untangling the tau gene association with neurodegenerative disorders. Hum Mol Genet. 2006;15(2):R188–95.
91. Rebeck GW, Hoe HS, Moussa CE. Beta-amyloid1-42 gene transfer model exhibits intraneuronal amyloid, gliosis, tau phosphorylation, and neuronal loss. J Biol Chem. 2010;285(10):7440–6.
92. Khandelwal PJ, et al. Parkinson-related parkin reduces alpha-Synuclein phosphorylation in a gene transfer model. Mol Neurodegener. 2010;5:47.
93. Hardy J. Expression of normal sequence pathogenic proteins for neurodegenerative disease contributes to disease risk: 'permissive templating' as a general mechanism underlying neurodegeneration. Biochem Soc Trans. 2005;33(Pt 4):578–81.
94. Myers AJ, et al. The MAPT H1c risk haplotype is associated with increased expression of tau and especially of 4 repeat containing transcripts. Neurobiol Dis. 2007;25(3):561–70.
95. Berger Z, et al. Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. J Neurosci. 2007;27(14):3650–62.
96. Gomez-Isla T, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol. 1997;41(1):17–24.
97. Rapoport M, et al. Tau is essential to beta-amyloid-induced neurotoxicity. Proc Natl Acad Sci U S A. 2002;99(9):6364–9.
98. Roberson ED, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science. 2007;316(5825):750–4.
99. Eskelinen E-L. Maturation of autophagic vacuoles in mammalian cells. Autophagy. 2005;1(1):1–10.
100. Kirkin V, et al. A role for ubiquitin in selective autophagy. Mol Cell. 2009;34(3):259–69.
101. Bjorkoy G, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol. 2005;171(4):603–14.
102. Tan JM, et al. Lysine 63-linked polyubiquitin potentially partners with p62 to promote the clearance of protein inclusions by autophagy. Autophagy. 2007;4(2):251–3.
103. Cheong H, et al. The Atg1 kinase complex is involved in the regulation of protein recruitment to initiate sequestering vesicle formation for nonspecific autophagy in Saccharomyces cerevisiae. Mol Biol Cell. 2008;19(2):668–81.
104. Geng J, et al. Quantitative analysis of autophagy-related protein stoichiometry by fluorescence microscopy. J Cell Biol. 2008;182(1):129–40.
105. Fukuda M, Itoh T. Direct link between Atg protein and small GTPase Rab: Atg16L functions as a potential Rab33 effector in mammals. Autophagy. 2008;4(6):824–6.

106. Hosokawa N, et al. Atg101, a novel mammalian autophagy protein interacting with Atg13. Autophagy. 2009;5(7):973–9.
107. Nair U, et al. Roles of the lipid-binding motifs of Atg18 and Atg21 in the cytoplasm to vacuole targeting pathway and autophagy. J Biol Chem. 2010;285(15):11476–88.
108. Geng J, Klionsky DJ. Determining Atg protein stoichiometry at the phagophore assembly site by fluorescence microscopy. Autophagy. 2010;6(1):144–7.
109. Mizushima N, et al. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell. 2004;15(3):1101–11.
110. Huang W-P, et al. The itinerary of a vesicle component, Aut7p/Cvt5p, terminates in the yeast vacuole via the autophagy/Cvt pathways. J Biol Chem. 2000;275(8):5845–51.
111. Kovács AL, Reith A, Seglen PO. Accumulation of autophagosomes after inhibition of hepatocytic protein degradation by vinblastine, leupeptin or a lysosomotropic amine. Exp Cell Res. 1982;137(1):191–201.
112. Shimura H, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet. 2000;25(3):302–5.
113. Kitada T, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998;392(6676):605–8.
114. Lucking CB, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. N Engl J Med. 2000;342(21):1560–7.
115. van de Warrenburg BP, et al. Clinical and pathologic abnormalities in a family with parkinsonism and parkin gene mutations. Neurology. 2001;56(4):555–7.
116. Mori H, et al. Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. Neurology. 1998;51(3):890–2.
117. Jellinger KA. Morphological substrates of mental dysfunction in Lewy body disease: an update. J Neural Transm Suppl. 2000;59:185–212.
118. Dawson TM, Dawson VL. The role of parkin in familial and sporadic Parkinson's disease. Mov Disord. 2010;25(Suppl 1):S32–9.
119. Imam SZ, et al. Novel regulation of parkin function through c-Abl-mediated tyrosine phosphorylation: implications for Parkinson's disease. J Neurosci. 2011;31(1):157–63.
120. Ko HS, et al. Phosphorylation by the c-Abl protein tyrosine kinase inhibits Parkin's ubiquitination and protective function. Proc Natl Acad Sci U S A. 2010;107(38):16691–6.
121. Rosen KM, et al. Parkin reverses intracellular beta-amyloid accumulation and its negative effects on proteasome function. J Neurosci Res. 2010;88(1):167–78.
122. Cookson MR, et al. RING finger 1 mutations in Parkin produce altered localization of the protein. Hum Mol Genet. 2003;12(22):2957–65.
123. Takahashi H, et al. Familial juvenile parkinsonism: clinical and pathologic study in a family. Neurology. 1994;44(3 Pt 1):437–41.
124. Sanchez MP, et al. Progressive supranuclear palsy and tau hyperphosphorylation in a patient with a C212Y parkin mutation. J Alzheimers Dis. 2002;4(5):399–404.
125. Morales B, et al. Steele-Richardson-Olszewski syndrome in a patient with a single C212Y mutation in the parkin protein. Mov Disord. 2002;17(6):1374–80.
126. Guerrero R, et al. Hyperphosphorylated tau aggregates in the cortex and hippocampus of transgenic mice with mutant human FTDP-17 Tau and lacking the PARK2 gene. Acta Neuropathol. 2009;117(2):159–68.
127. Moussa CE. Parkin attenuates wild-type tau modification in the presence of beta-amyloid and alpha-synuclein. J Mol Neurosci. 2009;37(1):25–36.
128. Rodriguez-Navarro JA, et al. Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. Neurobiol Dis. 2010;39(3):423–38.
129. Geisler S, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol. 2010;12(2):119–31.
130. Narendra D, et al. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol. 2008;183(5):795–803.

131. Park J, Kim Y, Chung J. Mitochondrial dysfunction and Parkinson's disease genes: insights from Drosophila. Dis Model Mech. 2009;2(7–8):336–40.
132. Vives-Bauza C, et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc Natl Acad Sci U S A. 2010;107(1):378–83.
133. Thomas KJ, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet. 2011;20(1):40–50.
134. Kanki T, et al. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. Dev Cell. 2009;17(1):98–109.
135. Novak I, et al. Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep. 2010;11(1):45–51.
136. Okamoto K, Kondo-Okamoto N, Ohsumi Y. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev Cell. 2009;17(1):87–97.
137. Wild P, Dikic I. Mitochondria get a Parkin' ticket. Nat Cell Biol. 2010;12(2):104–6.
138. Chen D, et al. Parkin mono-ubiquitinates Bcl-2 and regulates autophagy. J Biol Chem. 2010;285(49):38214–23.
139. Sutovsky P, et al. Ubiquitin tag for sperm mitochondria. Nature. 1999;402(6760):371–2.
140. Wang Y, et al. Synergy and antagonism of macroautophagy and chaperone-mediated autophagy in a cell model of pathological tau aggregation. Autophagy. 2010;6(1):182–3.
141. Dolan PJ, Johnson GV. A caspase cleaved form of tau is preferentially degraded through the autophagy pathway. J Biol Chem. 2010;285(29):21978–87.
142. Gonzalez-Polo RA, et al. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci. 2005;118:3091–102.
143. Dunn WA Jr. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. J Cell Biol. 1990;110(6):1923–33.
144. Ishihara N, et al. Autophagosome requires specific early Sec proteins for its formation and NSF/SNARE for vacuolar fusion. Mol Biol Cell. 2001;12(11):3690–702.
145. Reggiori F, et al. Early stages of the secretory pathway, but not endosomes, are required for Cvt vesicle and autophagosome assembly in Saccharomyces cerevisiae. Mol Biol Cell. 2004;15(5):2189–204.
146. Hebron ML, Lonskaya I, Moussa CE. Tyrosine kinase inhibition facilitates autophagic SNCA/alpha-synuclein clearance. Autophagy. 2013;9(8):1249–50.
147. Lonskaya I, et al. Tyrosine kinase inhibition increases functional parkin-Beclin-1 interaction and enhances amyloid clearance and cognitive performance. EMBO Mol Med. 2013;5(8):1247–62.
148. Hebron ML, Lonskaya I, Moussa CE. Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of alpha-synuclein in Parkinson's disease models. Hum Mol Genet. 2013;22(16):3315–28.
149. Lonskaya I, et al. Decreased parkin solubility is associated with impairment of autophagy in the nigrostriatum of sporadic Parkinson's disease. Neuroscience. 2013;232:90–105.
150. Lonskaya I, et al. Diminished parkin solubility and co-localization with intraneuronal amyloid-beta are associated with autophagic defects in Alzheimer's disease. J Alzheimers Dis. 2013;33(1):231–47.
151. Shibata M, et al. Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. J Biol Chem. 2006;281(20):14474–85.
152. Erlich S, Shohami E, Pinkas-Kramarski R. Neurodegeneration induces upregulation of Beclin 1. Autophagy. 2006;2(1):49–51.
153. Lonskaya I, et al. Nilotinib-induced autophagic changes increase endogenous parkin level and ubiquitination, leading to amyloid clearance. J Mol Med (Berl). 2014;92(4):373–86.
154. Lonskaya I, et al. Ubiquitination increases parkin activity to promote autophagic alpha-synuclein clearance. PLoS One. 2013;8(12):e83914.
155. Lonskaya I, et al. Decreased parkin solubility is associated with impairment of autophagy in the nigrostriatum of sporadic Parkinson's disease. Neuroscience. 2012;232C:90.

156. Kantarjian HM, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood. 2007;110(10):3540–6.
157. de Lavallade H, et al. Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. J Clin Oncol. 2008;26(20):3358–63.
158. Walden H, Martinez-Torres RJ. Regulation of Parkin E3 ubiquitin ligase activity. Cell Mol Life Sci. 2012;69(18):3053–67.
159. Stolz A, Ernst A, Dikic I. Cargo recognition and trafficking in selective autophagy. Nat Cell Biol. 2014;16(6):495–501.
160. Kondapalli C, et al. PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. Open Biol. 2012;2(5):120080.
161. Trempe JF, et al. Structure of parkin reveals mechanisms for ubiquitin ligase activation. Science. 2013;340(6139):1451–5.
162. Wauer T, Komander D. Structure of the human Parkin ligase domain in an autoinhibited state. EMBO J. 2013;32(15):2099–112.
163. Kane LA, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. J Cell Biol. 2014;205(2):143–53.
164. Koyano F, et al. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature. 2014;510(7503):162–6.
165. Kazlauskaite A, et al. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. Biochem J. 2014;460(1):127–39.
166. Kazlauskaite A, Muqit MM. PINK1 and Parkin—mitochondrial interplay between phosphorylation and ubiquitylation in Parkinson's disease. FEBS J. 2015;282(2):215–23.
167. Caulfield TR, et al. Phosphorylation by PINK1 releases the UBL domain and initializes the conformational opening of the E3 Ubiquitin Ligase Parkin. PLoS Comput Biol. 2014;10(11):e1003935.
168. Hebron ML, et al. Parkin ubiquitinates Tar-DNA binding protein-43 (TDP-43) and promotes its cytosolic accumulation via interaction with histone deacetylase 6 (HDAC6). J Biol Chem. 2013;288(6):4103–15.

Index

R. Clarke (ed.), *The Unfolded Protein Response in Cancer*, Cancer Drug Discovery and Development, https://doi.org/10.1007/978-3-030-05067-2