



ATF4 in cellular stress, ferroptosis, and cancer

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Abstract

Activating transcription factor 4 (ATF4), a member of the ATF/cAMP response element-binding (CREB) family, plays a critical role as a stress-induced transcription factor. It orchestrates cellular responses, particularly in the management of endoplasmic reticulum stress, amino acid deprivation, and oxidative challenges. ATF4's primary function lies in regulating gene expression to ensure cell survival during stressful conditions. However, when considering its involvement in ferroptosis, characterized by severe lipid peroxidation and pronounced endoplasmic reticulum stress, the ATF4 pathway can either inhibit or promote ferroptosis. This intricate relationship underscores the complexity of cellular responses to varying stress levels. Understanding the connections between ATF4, ferroptosis, and endoplasmic reticulum stress holds promise for innovative cancer therapies, especially in addressing apoptosis-resistant cells. In this review, we provide an overview of ATF4, including its structure, modifications, and functions, and delve into its dual role in both ferroptosis and cancer.

Keywords ATF4 · Ferroptosis · Cellular stress · Cancer

Introduction

Transcription factors play a role in regulating gene transcription rates by binding to DNA regulatory sequences, thereby maintaining essential cellular physiological processes. Among these factors, activator protein 1 (AP-1) was among the earliest mammalian transcription factors discovered, often as a dimeric complex (Bejjani et al. 2019). The activating transcription factor (ATF) family, a subset of AP-1, comprises seven members (Fig. 1): ATF1, ATF2, ATF3, ATF4, ATF5, ATF6, and ATF7. These members recognize and bind cyclic AMP response element-binding proteins (CREB), thereby regulating transcription rates of various target genes and contributing to intracellular homeostasis (Ameri and Harris 2008). Distinct sequence elements are bound by AP-1 transcription factors depending on unique

homodimer or heterodimer combinations, while ATF primarily binds to the sequence 5'-TGACGTCA-3' (Drust et al. 1991).

At first, identified as a transcriptional repressor of cAMP response elements, ATF4, also known as CREB2, demonstrates dual functionality, serving as both a transcriptional activator and repressor (Mielnicki and Pruitt 1991). It plays a multifaceted role in the regulation of various cellular biological processes and is induced for upregulation and activation, especially in response to stress conditions. While diverse extracellular signals can increase ATF4 levels in different cell types, the primary regulation occurs at the transcriptional initiation and post-transcriptional levels. The *ATF4* mRNA contains short upstream open reading frames (uORFs) within its 5' untranslated region (5'UTR) (Marasco et al. 2022). These uORFs encode "AUG" start codons and are typically suppressed under non-stimulatory conditions, resulting in reduced ATF4 protein synthesis. Conversely, under stress conditions, the assembly of the protein occurs, leading to the phosphorylation of the eukaryotic translation initiation factor 2 subunit alpha (EIF2S1) (Tameire et al. 2019). This event facilitates selective ATF4 translation, followed by its translocation to the nucleus for the regulation of gene transcription (Baniulyte et al. 2023; Gouveia Roque et al. 2023).

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	ATF1	ATF2	ATF3	ATF4	ATF5	ATF6	ATF7
Cellular localization	Nucleus	Nucleus Mitochondrial Cytosol	Nucleus	Nucleus	Nucleus Cytosol	Nucleus ER	Nucleus
Tissue specificity	Peripheral blood	Liver	Placenta	Platelet	Liver	Serum	Testis
Molecular mass	29.2 kD	54.5 kD	20.6 kD	38.6 kD	30.7 kD	74.6 kD	51.8 kD
Phosphorylation site	Ser63 Ser198	Ser62 Ser121 Ser490 Thr69 Thr71 Thr73	-	Ser215 Ser224 Ser235 Ser245 Ser248 Thr213	Ser92 Ser126 Ser190 Thr94	-	Thr51 Thr19

Fig. 1 Distinctions among ATF family members. The human ATF family encompasses ATF1, ATF2, ATF3, ATF4, ATF5, ATF6, and ATF7, each characterized by distinct molecular sizes, cellular localizations, tissue specificities, and phosphorylation sites

ATF4 demonstrates low expression in healthy cells, but its expression is elevated in cancer, where it can facilitate cell survival and tumor growth by inducing adaptive stress responses (Harding et al. 2003). For example, the transcriptional activation of ATF4 enhances the proliferation and invasiveness of gastric cancer cells (Wang et al. 2023). However, in cases of persistent stress and failed cellular homeostasis, ATF4 triggers apoptosis through pro-apoptotic factors, such as DNA damage-inducible transcript 3 (DDIT3) and tribbles pseudokinase 3 (TRIB3) (Ohoka et al. 2005). Moreover, cancer cells frequently exhibit metabolic aberrations, where metabolic reprogramming becomes associated with tumor progression, stress adaptation, and response to anti-tumor therapies (Luengo et al. 2017). For instance, disruptions in glutathione (GSH) metabolism are implicated in various malignant tumors' initiation, progression, and drug resistance (Miess et al. 2018; Wen et al. 2021). GSH serves as a scavenger, protecting cells from damage caused by reactive oxygen species (ROS) to essential cellular components, such as DNA, proteins, and lipids.

Ferroptosis, a form of iron-dependent regulated cell death, can be induced by GSH depletion and ROS accumulation (Dixon et al. 2012; Stockwell 2022; Stockwell et al. 2017). Certain cancer cells, due to their altered metabolism and ROS burden, display increased susceptibility to ferroptosis (Chen et al. 2022; Lin et al. 2022). For example, clear cell carcinoma constitutes a cluster of exceptionally aggressive malignancies marked by anomalous lipid and glycogen buildup. This leads to clear cell carcinoma cells that exhibit heightened susceptibility to ferroptosis (Zou et al. 2019). Pancreatic cancer has *KRAS* mutations, rendering it susceptible to ferroptosis despite the concurrent existence of established anti-ferroptotic mechanisms (Kuang et al. 2021; Li et al. 2021a, 2023). Consequently, targeting ferroptosis emerges as a promising strategy for tumor therapy (Chen

et al. 2021a; Dierge et al. 2021; Lei et al. 2022; Tang et al. 2023b; Zhang et al. 2023).

This review aims to provide a comprehensive understanding of the underlying mechanisms governing ATF4-mediated stress responses while also delving into its emerging involvement in ferroptotic cell death. A thorough elucidation of ATF4's regulation and function in the context of cell death offers potential avenues for the development of innovative therapeutic approaches in cancer treatment.

The mRNA and protein structure of ATF4

The ATF4 cDNA was initially isolated from human Jurkat cells and designated as tax-responsive enhancer element-binding protein 67 (TAXREB67) (Tsujiimoto et al. 1991). The human *ATF4* mRNA contains three short uORFs in the 5'UTR before the functional coding sequence, and these uORFs are crucial for ATF4's stress response (Harding et al. 2000). Adjacent to uORF1 in the 5' region lies a positive-acting element that facilitates ribosome scanning and commencement of coding downstream from the *ATF4* mRNA (Xiao et al. 2022). Positioned in the middle is uORF2, functioning as a repressor element that curbs ATF4 expression. Sequentially following uORF2 is the ATF4 ORF, a crucial segment for ATF4 expression regulation. Normally, EIF2S1 activation governs the translatability of *ATF4* mRNA. However, it has been reported that non-coding RNAs can also impact ATF4 activation by influencing the stability of *ATF4* mRNA. For instance, the non-coding RNA circBtl1 reduces the self-renewal capacity of intestinal stem cells by destabilizing *ATF4* mRNA (Guo et al. 2023). Hence, stabilizing *ATF4* mRNA and activating EIF2S1 are crucial prerequisites for increasing ATF4 protein levels.

The ATF4 protein consists of 351 amino acids, featuring distinct structural domains and motifs, including basic leucine zipper (bZIP) domains. These bZIP domains facilitate interactions with other bZIP factors to form homodimers or heterodimers, playing a crucial role in DNA binding and enhancing ATF4 stability (Yin et al. 2017). The ATF4 bZIP domain comprises a base region for DNA binding and a leucine zipper for dimerization. Stress-induced ATF4 regulation relies on motif coordination for protein stability. Additionally, ATF4 has an activating N-terminal domain that interacts with other proteins to regulate gene transcription (Schoch et al. 2001). This domain partners with genetic regulatory elements to control target gene transcription during stress, impacting stress response mechanisms.

Translational control of ATF4 synthesis

Phosphorylation of EIF2S1 is a critical step to enhance ATF4 mRNA translation. Upstream kinases including eukaryotic translation initiation factor 2 alpha kinase 1 (EIF2AK1), eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2), eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3), and eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4) respond to stress and phosphorylate EIF2S1 at Ser51 (Fessler et al. 2020). These kinases are activated by distinct triggers: EIF2AK1 by heme deficiency, EIF2AK2 by viral infection, EIF2AK3 by ER stress, and EIF2AK4 by amino acid deficiency (Fessler et al. 2020).

EIF2S1 is a subunit of the eukaryotic translation initiation factor, crucial for initiating protein synthesis. When phosphorylated, EIF2S1 acts as a powerful inhibitor of EIF2S2, binding to alternative sites and interrupting the exchange of guanosine-5'-diphosphate (GDP) for guanosine triphosphate (GTP) during EIF2 complex activation (Adomavicius et al. 2019; Zyryanova et al. 2021). This exchange is crucial for assembling the 43S ribosome. As a result, phosphorylated EIF2S1 reduces the GDP-GTP exchange factor, leading to decreased protein synthesis (Adomavicius et al. 2019). However, mammalian *ATF4* mRNA contains an inhibitory uORF that overlaps with the *ATF4* coding ORF (Vattem and Wek 2004). In cells with abundant EIF2-GTP, the inhibitory ORF initiates translation, bypassing the coding ORF. Conversely, when EIF2-GTP levels are low, translation bypasses the inhibitory ORF, allowing *ATF4* coding ORF translation to commence. This suggests that ATF4 protein may be upregulated when EIF2S1 is phosphorylated and EIF2-GTP levels are diminished.

Post-translational modification of ATF4 protein

ATF4 undergoes various post-translational modifications that impact its stability and transcriptional activity. Ubiquitination plays a role in regulating ATF4's stability. A proteomics analysis has identified multiple lysine residues in ATF4 that have the potential to be ubiquitinated, although experimental confirmation is pending for many of these sites (Hornbeck et al. 2015; Kim et al. 2011). Within the nucleus, ATF4 interacts with beta-transducin repeat-containing E3 ubiquitin-protein ligase (BTRC), an F-box protein involved in the SCF E3 ubiquitin ligase complex. Subsequent research has highlighted that this ubiquitination process, initiated by BTRC, leads to ATF4's degradation via the ubiquitin-proteasome pathway, and this degradation is contingent on ATF4 phosphorylation at Ser219 (Frank et al. 2010; Lassot et al. 2001).

Moreover, egl-9 family hypoxia-inducible factor 3 (EGLN3) can prompt ATF4 protein degradation by catalyzing proline hydroxylation within ATF4's oxidation-dependent degradation domain (Köditz et al. 2007). However, another perspective suggests that ATF4 interacts with EGLN2 and EGLN3, but it's EGLN2 that stabilizes ATF4 without causing hydroxylation (Hiwatashi et al. 2011). Despite ATF4 stabilization by EGLN2, its transcriptional activity is repressed. This suggests a nuanced relationship between ATF4 protein levels and its transcriptional function. Another regulatory mechanism involves the histone acetyltransferase E1A binding protein P300 (EP300), which directly binds to ATF4's N-terminus to stabilize it, potentially by preventing ubiquitination at multiple sites and thwarting proteasomal degradation (Lassot et al. 2005).

While regulating ATF4 stability is crucial, equal attention must be given to its transcriptional activity. Within B-cell progenitors and cardiomyocytes, the protein arginine methyltransferase 1 (PRMT1) amplifies ATF4's transcriptional capabilities by methylating the Arg239 site. This modification is facilitated by the BTG anti-proliferation factor 1 (BTG1) (Kim et al. 2022b; Yuniati et al. 2016). Furthermore, ATF4's transcriptional activity is influenced by phosphorylation events. For instance, ribosomal protein S6 kinase A3 (RPS6KA3)-mediated phosphorylation of ATF4 Ser245 in osteoblasts boosts its transcriptional competence (Yang et al. 2004). Casein kinase 2 (CSNK2) catalyzes phosphorylation of ATF4's Ser215 site, promoting transcription of ATF3 and DDIT3—genes within ATF4's scope (Ampofo et al. 2013). CSNK2's influence extends to DDIT3, where its phosphorylation curbs the gene's activity (Schwind et al. 2015). This duality of phosphorylation's impact on ATF4 is evident in the context

of the Ret proto-oncogene (RET), which phosphorylates ATF4 at Thr107, 114, 115, and 109, ultimately dampening ATF4's transcriptional potency (Bagheri-Yarmand et al. 2015). Consequently, this leads to reduced transcript levels of phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1) and BCL2 binding component 3 (BBC3) (Bagheri-Yarmand et al. 2015). Such instances highlight the potential divergence in transcriptional activity and target genes contingent upon ATF4's phosphorylation site.

Exploring ATF4's post-translational modifications is crucial due to numerous undisclosed modification sites. For example, EP300 acetylates ATF4 at Lys311, with its stabilizing effect being independent of acetylation function (Lassot et al. 2005). This highlights potential unexplored roles for acetylation within ATF4's bZIP domain.

In summary, investigating ATF4's post-translational modifications is essential, considering the many sites with unknown functions.

ATF4 and cellular stress

The phosphorylation of EIF2S1 at the Ser51 site stands as a pivotal event in diverse stress signaling pathways. While generally inhibiting protein synthesis, this phosphorylation uniquely triggers the translation of specific mRNAs, including ATF4. This phosphorylation is catalyzed by four enzymes: EIF2AK1 (induced by heme deficiency or oxidative stress), EIF2AK2 (viral infection), EIF2AK3 (amino acid deficiency and ultraviolet light), and EIF2AK4 (ER stress and hypoxia), acting independently or cooperatively. Consequently, ATF4 can be upregulated in response to distinct cell stress types (ER stress, oxidative stress, amino acid depletion, integrated stress response [ISR]) (Fig. 2), functioning as both a transcriptional activator and inhibitor.

ATF4 exerts its regulatory influence on target gene transcription by binding to C/EBP-ATF response element (CARE) sequences, which facilitate transcriptional activation in response to diverse stresses (Fawcett et al. 1999).

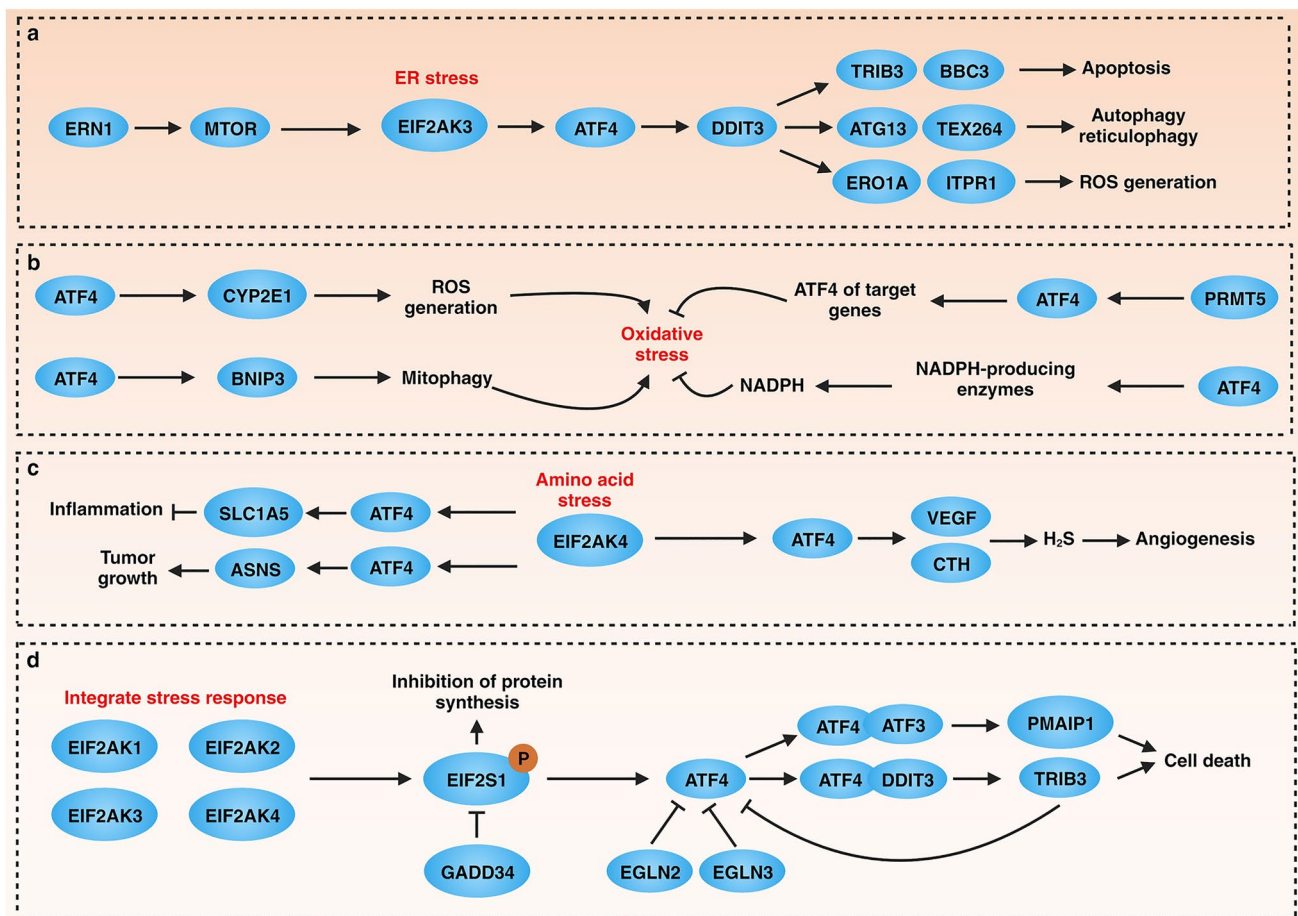


Fig. 2 The role of ATF4 in cell stress. In response to various cellular stressors, ATF4 can be upregulated and activated by four kinases (EIF2AK1, EIF2AK2, EIF2AK3, and EIF2AK4). As a result, ATF4

serves as a crucial effector molecule in multiple stress pathways: endoplasmic reticulum stress (a), oxidative stress (b), amino acid stress (c), and integrated stress response (d)

Originally observed in surrogate rat fibroblasts, *ATF4* mRNA levels were induced by hypoxia (Estes et al. 1995). However, ATF4's role in cellular stress response varies based on stimulus intensity, type, and timing, as elaborated below.

ER stress

The ER serves as a central hub for protein synthesis, folding, and modifications. Disruptions caused by external factors or internal events can impair ER's protein folding capacity, leading to an accumulation of misfolded proteins and perturbations in protein homeostasis, culminating in ER stress (Kohli et al. 2021). The unfolded protein response (UPR) is activated in response to ER stress, forming a complex network of signaling pathways that orchestrate adaptive reactions.

This UPR comprises three distinct pathways, each activated by a specific transmembrane sensor: endoplasmic reticulum to nucleus signaling 1 (ERN1), EIF2AK3, and ATF6. These sensors, in their native state, associate with the chaperone heat shock protein family A member 5 (HSPA5), preventing downstream signaling activation. However, when misfolded proteins accumulate, HSPA5's higher affinity for these proteins prompts its dissociation from the sensors, thus triggering the unfolded protein response. ERN1 can bind to misfolded proteins, also activating the UPR (Gardner and Walter 2011). These pathways collectively reduce unfolded protein burden by attenuating protein synthesis, augmenting ER's protein folding capacity, and enhancing ER-associated degradation (ERAD). Nevertheless, if the UPR fails to effectively restore protein homeostasis, it can trigger cell death signals.

During ER stress, ATF4 activation hinges on the EIF2AK3-EIF2S1 axis (Fig. 2a), which subsequently prompts ATF4 to induce expression of DDIT3 and several other related proteins involved in autophagy, antioxidant defense, and cell death pathways (Huang et al. 2021; Wei et al. 2022; Zielke et al. 2021). As the primary downstream effector of ATF4, DDIT3 upregulates pro-apoptotic proteins including TNF receptor superfamily member 10b (TNFRSF10B), TRIB3, BCL2 like 11 (BCL2L11), and BBC3, while suppressing the expression of the anti-apoptotic protein BCL2 (Wang and Kaufman 2016). Moreover, within breast cancer cells, DDIT3 triggers the activation of endoplasmic reticulum oxidoreductase 1 alpha (ERO1A). This, in turn, enhances 1,4,5-trisphosphate inositol receptor type 1 (ITPR1)-mediated ER calcium release to mitochondria, leading to heightened ROS production (Varone et al. 2021).

ERN1 can indirectly influence ATF4 activation. Mechanically, ERN1, activated by ER stress in chondrocytes, triggers apoptosis through the mechanistic target of rapamycin kinase (MTOR)-EIF2AK3-ATF4-DDIT3 pathway (Li et al.

2021e). The EIF2AK3-ATF4 pathway is crucial for ATF6 activation in mouse liver (Teske et al. 2011), indicating ATF4's significance in coordinating the functions of three ER transmembrane sensors.

Recently, a phenomenon known as reticulophagy, which involves the selective degradation of the ER through autophagy, has been discovered during ER stress. ATF4 plays a key role as a regulator bridging ER stress and reticulophagy. Glioblastoma cells treated with loperamide (LOP) exhibit increased ATF4 expression along with ER stress and reticulophagy induction (Zielke et al. 2021). Upregulation of ATF4-dependent autophagy genes, such as autophagy-related 13 (*ATG13*), WD repeat domain phosphoinositide interacting 1 (*WIPI1*), microtubule-associated protein 1 light chain 3 beta (*MAP1LC3B*), GABA type A receptor-associated protein (*GABARAP*), testis-expressed 264 (*TEX264*), and reticulophagy regulator 1 (*RETREG1*), becomes crucial for autophagy and reticulophagy (Zielke et al. 2021). Yet, it is still unclear whether ATF4 specifically triggers ER stress-induced reticulophagy.

Oxidative stress

Cellular redox control hinges on maintaining a delicate equilibrium between the generation of ROS/reactive nitrogen species (RNS) and the intrinsic defense mechanisms provided by antioxidants. These mechanisms are crucial for virtually all cellular activities. Alongside direct antioxidants, cells rely on indirect antioxidant systems to either curtail ROS/RNS formation or neutralize their active byproducts. When ROS/RNS production outpaces antioxidant capacity, oxidative stress emerges, prompting distinct cellular responses. Two vital intracellular antioxidants, GSH and thioredoxin (TXN), heavily rely on nicotinamide adenine dinucleotide phosphate (NADPH) to sustain their reduced states. Optimal levels of ROS play a role in fostering cell proliferation and viability. NADPH oxidase 4 (NOX4) stimulates NFE2-like BZIP transcription factor 2 (NFE2L2)-PINK1-mediated mitophagy by producing mitochondrial ROS, thus promoting the survival of hepatocellular carcinoma (Peng et al. 2023). Conversely, excessive ROS can trigger detrimental effects, such as protein and lipid peroxidation, DNA damage, and cell demise (Liu et al. 2022; Yu et al. 2021).

Like NFE2L2 (Dai et al. 2020a; Lane et al. 2023; Sun et al. 2016a, 2016b; Wang et al. 2021), ATF4 plays a role in regulating various oxidative stress processes (Fig. 2b). It is typically upregulated in response to oxidative stress, where it oversees the expression of multiple genes, including those responsible for antioxidant enzymes, heat shock proteins, and redox-related factors. This regulatory activity helps mitigate oxidative stress-induced damage and maintains intracellular redox homeostasis. Consequently, inhibiting

intracellular ATF4 can elevate the risk of oxidative stress. For example, in acute myeloid leukemia cases, the inhibition of protein arginine methyltransferase 5 (PRMT5) leads to the expression of unstable *ATF4* mRNA with retained introns, causing nuclear stalling (Szewczyk et al. 2022). Simultaneously, reduced cytoplasmic ATF4 splice transcription results in decreased expression of ATF4 target genes and subsequent oxidative stress (Szewczyk et al. 2022).

Intracellular antioxidants rely on NADPH for their effectiveness, and ATF4 can stimulate NADPH production through various pathways. In mouse cardiomyocytes, ATF4 emerges as a regulatory factor governing the upregulation of essential enzymes (e.g., G6PDX, PHGDH, PSAT1, and MTHFD2) within both NADPH production pathways (Wang et al. 2022b). Overexpressing ATF4 amplifies NADPH enzyme production, effectively safeguarding against oxidative stress-triggered heart failure (Wang et al. 2022b). This antioxidative prowess of ATF4 holds therapeutic potential, as demonstrated by its role in mitigating oxidative stress in erythrocytes through the EIF2AK1-EIF2S1-ATF4 pathway (Suragani et al. 2012). This pathway also proves indispensable for erythroid differentiation, highlighting ATF4's promise as a therapeutic avenue for addressing β -thalassemia (Suragani et al. 2012).

While ATF4 primarily acts as an adaptive response to oxidative stress, it can also initiate or enhance oxidative stress in some cases. For instance, in primary hepatocytes, ATF4 silencing impedes palmitate-triggered ROS generation and upregulation of cytochrome P450 family 2 subfamily E member 1 (CYP2E1) (Wang et al. 2014). ATF4 mediates CYP2E1 expression through a CREB-dependent mechanism and directly activates CYP2E1 promoter activity (Wang et al. 2014). This ATF4-driven ROS production can be curtailed by using CYP2E1 inhibitors. Furthermore, during cadmium-induced oxidative stress in human placental trophoblast cells, ATF4 can be activated by EIF2AK4, prompting BCL2 interacting protein 3 (BNIP3)-dependent mitochondrial autophagy (Zhu et al. 2021). This underscores ATF4's potential role as a central mediator connecting oxidative stress and autophagy.

Amino acid stress

At the cellular level, dietary protein restriction triggers amino acid deprivation, setting off amino acid response signaling pathways (Tabata et al. 2023). The EIF2AK4 kinase acts as a sensor by binding uncharged tRNAs, which prompts EIF2S1 phosphorylation. EIF2AK4's kinase activity is engaged upon interaction with any uncharged tRNA molecule (Wek et al. 1995). Consequently, the depletion of any single amino acid at the cellular level instigates the amino acid response. During amino acid abundance, ATF4's two uORFs engage with ribosomal machinery, preventing

ATF4's core coding region translation and thus maintaining low ATF4 levels (Vattem and Wek 2004). Conversely, amino acid scarcity triggers EIF2S1 phosphorylation by EIF2AK4. This allows the 40S ribosomal subunit to bypass ATF4's second uORF before binding to the ATF4 coding region, increasing ATF4 expression (Vattem and Wek 2004).

ATF4 is a key player in the amino acid response, capable of binding all recognized amino acid response element (AARE) sequences (Fig. 2c) (Siu et al. 2002). The EIF2AK4-ATF4 pathway is activated in sulfur-containing amino acid-deficient endothelial cells, where ATF4 induces vascular endothelial growth factor (VEGF) expression, influences glucose utilization via glycolysis and the pentose phosphate pathway, and spurs endothelial migration and proliferation through hydrogen sulfide production driven by cystathionine gamma-lyase (CTH) (Longchamp et al. 2018). Conversely, in intestinal epithelial cells, ATF4 deficiency hampers glutamine uptake and reduces antimicrobial peptide expression by downregulating solute carrier family 1 member 5 (SLC1A5) transcription (Hu et al. 2019). However, not all amino acid deficits engage the EIF2AK4-ATF4 axis; leucine deficiency prompts hemoglobin production in erythrocytes through the MTOR-EIF4EBP1 axis rather than the EIF2AK4-ATF4 axis (Chung et al. 2015).

Tumor cells frequently undergo mutations to modulate amino acid metabolism since they necessitate amino acids and other macromolecular precursors for sustaining growth (Vander Heiden et al. 2009). In *KRAS*-mutated cancer cells, ATF4 regulates the expression of crucial amino acid transport proteins, maintaining intracellular amino acid levels (Gwinn et al. 2018). Upon amino acid deprivation, *KRAS* mutation elevates *ATF4* mRNA levels through PI3K-AKT-mediated NFE2L2 upregulation (Gwinn et al. 2018). EIF2AK4-ATF4 activation further boosts ATF4 translation, augmenting ATF4 target gene expression including asparagine synthetase (*ASNS*), thereby promoting tumor growth (Gwinn et al. 2018). Gaining a more comprehensive understanding of ATF4's involvement in diverse amino acid metabolism processes will pave the way for innovative approaches in treating a wide range of diseases (McIntyre et al. 2023).

Integrated stress response

The ISR is a highly conserved network of intracellular signals that orchestrates adaptive responses to diverse environmental changes, safeguarding cellular and organismal well-being. This response can be triggered by various distinct stressors, encompassing ER stress, amino acid deficiency, heme scarcity, viral infection, and, notably in cancer biology, oncogene activation (Gwinn et al. 2018). This intricate mechanism operates through the activation of four specific kinases (EIF2AK1, EIF2AK2, EIF2AK3, and EIF2AK4),

each inducing the phosphorylation of the translation initiation factor EIF2S1 under specific stress contexts. Despite sharing significant kinase catalytic domain homology, these kinases boast distinct regulatory domains (Berlanga et al. 1998; Chen et al. 1991). EIF2S1 activation occurs via dimerization and autophosphorylation within all four kinases. Once phosphorylated, EIF2S1 hinders general protein synthesis while intriguingly amplifying the translation of select mRNA species, including ATF4 and protein phosphatase 1 regulatory subunit 15A (PPP1R15A, also known as GADD34). GADD34 participates in a feedback mechanism that directs protein phosphatase 1 (PP1) to dephosphorylate phospho-EIF2S1, enabling renewed protein synthesis and effectively culminating the ISR (Novoa et al. 2001).

The defining feature of the ISR is the elevation and activation of ATF4 (Fig. 2d) (Harding et al. 2000). This response, when engaged at low or short-term levels, typically steers a pro-survival pathway. However, under prolonged or intense activation, it can drive cell death (Andrysik et al. 2022). Cellular outcomes depend on ATF4's regulation of target genes through complex mechanisms involving transcription, translation, post-translation, and interactions with other transcription factors. Despite common mediators, the ISR elicits distinct responses to different cellular stresses. ATF4 target gene activation is influenced by stress severity, cellular context, and stimulus duration. For instance, renal epithelial cells respond to oxidative stress and amino acid deprivation with ISR-ATF4 activation in a scenario resembling amino acid scarcity due to tricarboxylic acid cycle inhibition (Ryan et al. 2021). Suppressing *ATF4* via siRNA leads to diminished protein and transcript levels of ATF4 target genes, perturbed GSH synthesis, and disrupted amino acid levels (Ryan et al. 2021).

While ATF4 primarily serves as a transcriptional activator for a cohort of genes implicated in the ISR, it can also exert transcriptional repression effects. This multifaceted behavior is demonstrated in the mouse hippocampus, where heightened phosphorylation of ATF4's Ser219 residue leads to ubiquitination-mediated degradation (Smith et al. 2020). This degradation liberates CREB, fostering enhanced CREB-dependent gene expression (Smith et al. 2020). Additionally, ATF4 can indirectly enforce inhibitory effects. For instance, in mouse neuronal cells, ATF4 partners with the DISC1 scaffold protein to facilitate phosphorylation of DISC1 at Ser58, consequently inhibiting phosphodiesterase 4D (PDE4D) via mutual binding (Soda et al. 2013). ATF4 doesn't engage directly with PDE4D in this context.

While unbound to the DNA of target genes, ATF4 exists in a monomeric form (Podust et al. 2001). However, ATF4's transcriptional specificity is influenced by its ability to form heterodimers with other bZIP or AP-1 members, thereby modulating the outcomes of ISR signaling. In multiple myeloma cells, ATF4 forges a complex with

ATF3, capable of binding to the PMAIP1 promoter, thereby activating PMAIP1 transcription (Wang et al. 2009). In the *Drosophila* model, ATF4's interaction with DDIT3 triggers *TRIB3* expression, ultimately triggering cell death (Ohoka et al. 2005). Some of ATF4's binding partners can repress its transcriptional activity. In HeLa cells, EGLN2 and EGLN3 associate with ATF4, curbing its transcriptional potency amidst hypoxia (Hiwatashi et al. 2011; Köditz et al. 2007). This dynamic also extends to the *Drosophila* ISR, where *TRIB3* interaction curtails ATF4's transcriptional vigor. *TRIB3* also stands as an ATF4 target gene, presenting an intricate negative feedback loop, intricately fine-tuning ISR (Wang et al. 2009).

Moreover, the regulatory landscape of the ISR isn't confined to ATF4's transcriptional control alone. Obg like ATPase 1 (OLA1) in cancer cells influences ISR regulation (Chen et al. 2015). OLA1, belonging to the ancient Obg GTPase family, acts as an eIF2 regulatory protein, impeding protein synthesis and promoting ISR and ATF4 production by binding eIF2, catalyzing GTP hydrolysis, and disrupting ternary complex formation (Chen et al. 2015).

Overall, the future of ISR and ATF4 research lies in uncovering the intricate details of their functions, interactions, and regulatory mechanisms, which could ultimately lead to new therapeutic strategies and a deeper understanding of cellular stress responses.

ATF4 in ferroptosis

Ferroptosis was initially identified as a non-apoptotic cell death pathway, primarily revealed through the screening of compounds, such as erastin and RSL3 (Dixon et al. 2012; Liu et al. 2021a). These compounds demonstrated selective lethality towards cancer cells that harbor mutant *RAS* oncogenes (Chen et al. 2021c). In contrast to apoptosis, ferroptosis exhibits distinct biochemical and morphological characteristics (Tang et al. 2021; Xie et al. 2016). Morphologically, ferroptosis involves cellular swelling and plasma membrane disruption, while apoptosis is characterized by cytoplasmic and nuclear condensation with an intact cell membrane. A defining feature of ferroptosis is its reliance on iron-driven lipid peroxidation, which operates independently of the caspases central to apoptosis (Lin et al. 2021). This unique mechanism entails perturbed iron ions, lipid metabolism, and redox systems, ultimately resulting in organelle and cellular membrane deterioration (Wu et al. 2021). The pivotal player in suppressing ferroptosis within the antioxidant system is glutathione peroxidase 4 (GPX4) (Han et al. 2021a; Li et al. 2023; Xie et al. 2023b; Xue et al. 2023a; Yang et al. 2014). Nevertheless, GPX4-independent pathways also demonstrate context-dependent roles in inhibiting lipid peroxidation.

ATF4 modulates ferroptosis sensitivity through multiple pathways, including interactions with other transcription factors (He et al. 2023; Lane et al. 2023; Sun et al. 2016b; Zhu et al. 2017) (Fig. 3). The heightened expression of ATF4 plays a dual role in ferroptosis, depending on its regulation of target genes, as discussed below.

ATF4 inhibits ferroptosis

HSPA5 is a chaperone protein induced by ER stress (Cerezo and Rocchi 2017). Its roles span signaling control, proliferation, invasion, apoptosis, inflammation, and immunity (Cerezo and Rocchi 2017). In the context of human pancreatic ductal adenocarcinoma (PDAC), erastin triggers a dose-dependent elevation of HSPA5 protein expression (Zhu et al. 2017). In contrast, *ATF4*-knockdown prevents the upregulation of HSPA5 (Zhu et al. 2017). HSPA5, in turn, enhances GPX4 protein stability, contributing to ferroptosis resistance through their interaction (Zhu et al. 2017). Targeting the ATF4-HSPA5-GPX4 axis enhances gemcitabine sensitivity in PDAC and provides a potential strategy against drug resistance in pancreatic cancer. This axis-mediated

ferroptosis resistance extends to gliomas, glial cells, and carp hepatocytes (Chen et al. 2019; Cui et al. 2022; Shao et al. 2023). However, mesencephalic astrocyte-derived neurotrophic factor (MANF) attenuates ferroptosis-driven lung injury by diminishing ER stress and restraining HSPA5-EIF2AK3-ATF4 signaling in lung tissue (Zeng et al. 2023). Yet, the precise mechanism behind this phenomenon awaits elucidation.

Solute carrier family 7 member 11 (SLC7A11), a component of the cystine/glutamate reverse transporter protein vital for GSH synthesis, plays a pivotal role as a negative regulator of ferroptosis (Dixon et al. 2012, 2014). In ER stress-prone MUP-uPA mouse hepatocytes, *ATF4* deficiency leads to diminished hepatic steatosis but heightened vulnerability to ferroptosis, culminating in accelerated hepatocellular carcinoma progression (He et al. 2023). Intriguingly, introducing SLC7A11 via ectopic expression reduces ferroptosis susceptibility and hepatocarcinogenesis (He et al. 2023). Thus, ATF4-dependent SLC7A11 expression inhibits ferroptosis.

Thioredoxin domain-containing protein 12 (TXNDC12) is an ER protein involved in redox signaling and regulation.

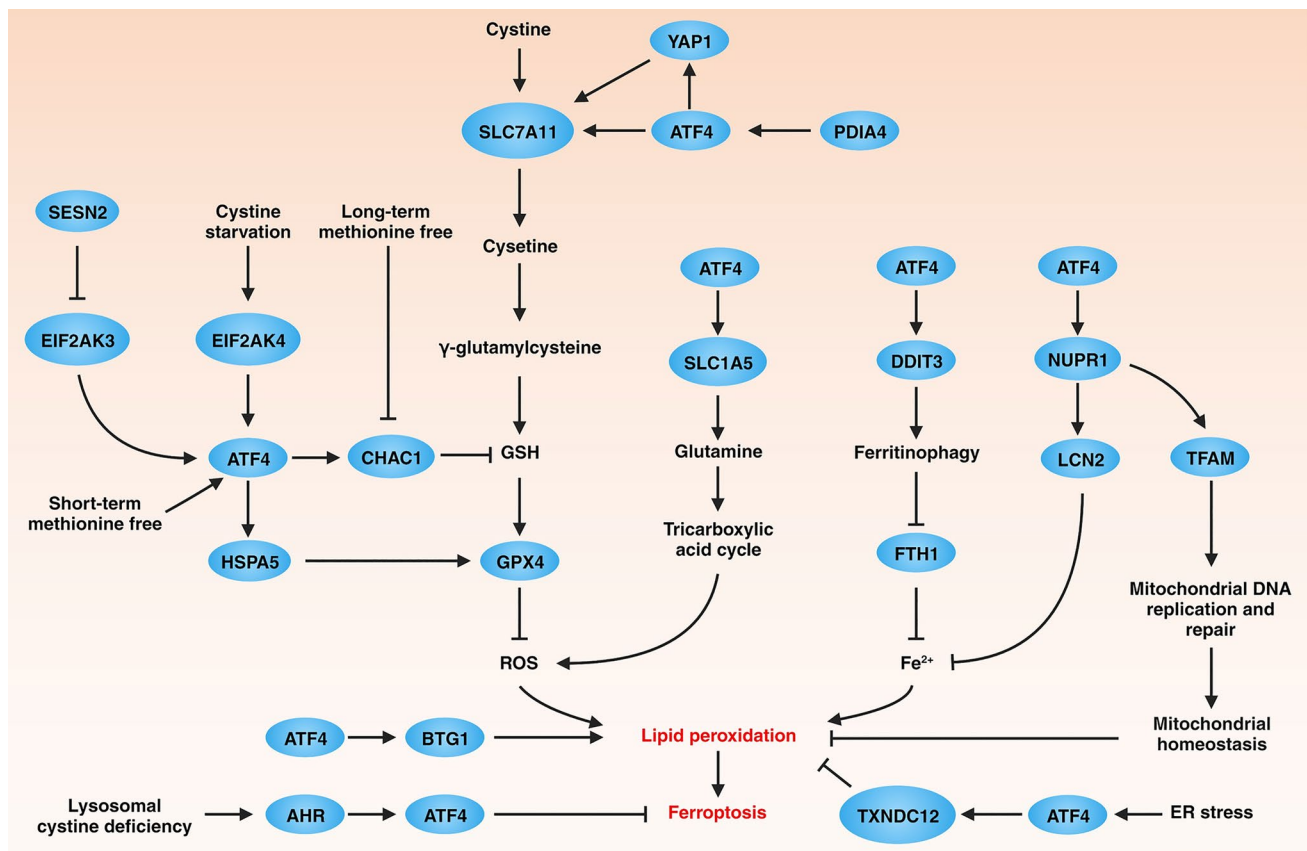


Fig. 3 The role of ATF4 in ferroptosis. ATF4 exerts its influence on ferroptosis through a variety of pathways. Several target genes of ATF4, including *HSPA5*, *SLC7A11*, and *NUPR1*, act as negative reg-

ulators of ferroptosis. Conversely, other ATF4 target genes, such as *BTG1*, *DDIT3*, *CHAC1*, and *SLC1A5*, positively regulate ferroptosis

This protein contains a thioredoxin domain, which is associated with the regulation of cellular redox homeostasis and plays a role in the reduction of disulfide bonds in other proteins. In leukemia cells, ER stress triggered by erastin or RSL3 activates ATF4, rather than NFE2L2, resulting in the upregulation of TXNDC12 transcription and the suppression of lipid peroxidation in a GPX4-independent manner (Lanlan et al. 2023). Therefore, directing interventions towards TXNDC12 presents a promising strategy for overcoming ferroptosis resistance in leukemia cells.

Another key player, protein disulfide isomerase family A member 4 (PDIA4), an ER enzyme, orchestrates protein disulfide bond formation, breaking, and rearrangement, critical for protein folding (Lee et al. 2022). In renal cell carcinoma, salinomycin induces autophagic degradation of PDIA4, concurrently attenuating the EIF2AK3-ATF4-SLC7A11 signaling pathway (Kang et al. 2023). This inhibition, in turn, hinders the GPX4-mediated defense against lipid peroxidation, ultimately promoting ferroptosis (Kang et al. 2023).

YAP/TAZ, pivotal effectors of Hippo signaling, modulate diverse cellular functions, including tumorigenesis and tissue development (Ma et al. 2022; Wang et al. 2022a). Despite previous findings indicating that YAP/TAZ sensitizes cells to ferroptosis via increased expression of NADPH oxidase 4 (NOX4), acyl-CoA synthetase long-chain family member 4 (ACSL4), and transferrin receptor (TFRC) (Wu et al. 2019; Yang et al. 2019), a recent study uncovered a contrasting role for YAP/TAZ in hepatocellular carcinoma. YAP/TAZ emerged as a negative regulator of ferroptosis, orchestrating ATF4-dependent induction of SLC7A11 expression via TEA domain transcription factor (TEAD) (Gao et al. 2021). Mechanistically, YAP/TAZ collaborates with ATF4, facilitating ATF4's nuclear entry and preventing cytoplasmic degradation. Inside the nucleus, ATF4 binds to DNA fragments within the AARE region of the *SLC7A11* promoter, orchestrating SLC7A11 expression (Gao et al. 2021). Thus, these findings establish a feedback loop between Hippo signaling and ATF4 activation to control SLC7A11 expression during ferroptosis.

Moreover, ATF4 unveils additional avenues for SLC7A11 upregulation. In glutamine and cystine-starved melanomas, ATF4 induces γ -glutamylcyclotransferase 1 (CHAC1) expression, reducing GSH levels and activating the NFE2L2-SLC7A11 axis (Kreß et al. 2023). Additionally, ATF4 directly activates NFE2L2, promoting the expression or production of SLC7A11, GSH, and NADPH (Kress et al. 2023). The G-quadruplex is a secondary nucleic acid structure with three stacks of G-tetrads. In the *NFE2L2* mRNA 5' untranslated region, it regulates de novo NFE2L2 protein translation under oxidative stress. Additionally, the formation of G-quadruplex structures in liver cancer cells enhances ATF4 expression during erastin-induced ferroptosis (Xie et al.

2023a). Taken together, this intricate network highlights the presence of adaptive mechanisms within the ISR, resulting in the activation of ATF4 and NFE2L2.

Cysteine plays a vital role in regulating GSH synthesis, and a recent report has revealed that the AHR-ATF4 axis mediates cysteine responses in lysosomes, influencing ferroptosis sensitivity (Swanda et al. 2023). Mechanistically, lysosomal cystine depletion activates the aromatic hydrocarbon receptor (AHR), leading to its nuclear translocation. Within the nucleus, AHR binds to the *ATF4* promoter, inducing *ATF4* expression, thus reducing susceptibility to ferroptosis. This pathway operates independently of the conventional ISR and oxidative stress, governing ATF4's transcriptional regulation in the nucleus (Swanda et al. 2023).

ATF4 also plays an anti-ferroptotic role in promoting nuclear protein 1 (NUPR1) expression. NUPR1 was initially identified as a stress-responsive protein during pancreatitis, pancreatic development, and regeneration (Mallo et al. 1997), with its regulation being under the control of ATF4 (Jin et al. 2009). In human and murine PDAC cells, NUPR1 plays a protective role against ferroptosis by directly increasing lipocalin 2 (LCN2) expression, a process dependent on ATF4 activation (Liu et al. 2021b). LCN2 plays a crucial role in regulating iron homeostasis by inhibiting iron uptake, thereby limiting the host's ability to acquire iron from the cellular environment. In addition to regulating LCN2 expression, the NUPR1 inhibitor ZZW-115 induces mitochondria-dependent ferroptosis in PDAC and hepatocellular carcinoma cells. Inhibition of NUPR1 by ZZW-115 represses transcription factor A mitochondrial (TFAM), leading to ROS generation and lipid peroxidation due to mitochondrial dysfunction (Huang et al. 2021). This complex interplay highlights NUPR1 as a transcriptional effector modulated by ATF4, potentially influencing ferroptosis defense mechanisms through multiple pathways. Similarly, tomatidine, a known compound for preventing muscle wasting, can disrupt ATF4-dependent signaling to induce ferroptosis, thus impeding the progression of pancreatic cancer (Mukherjee et al. 2023).

ATF4 promotes ferroptosis

In specific contexts, ATF4 can promote ferroptosis by modulating the expression of genes, such as DDIT3 and CHAC1, in HT1080 or retinal pigment epithelial cells upon exposure to erastin (Dixon et al. 2014; Sun et al. 2018). CHAC1, originally identified as upregulated during ER stress induced by DDIT3 (Mungrue et al. 2009), selectively impacts GSH by cleaving the γ -glutamyl bond of GSH (Kumar et al. 2012). The direct connection between CHAC1 and ferroptosis is established due to its involvement in erastin-induced ferroptosis and ER stress (Dixon et al. 2014). The EIF2AK4-ATF4-CHAC1 signaling pathway also plays a pivotal role

in ferroptosis and necroptosis triggered by cystine starvation in triple-negative breast cancer (Chen et al. 2017). *CHAC1*-knockdown rescues the decrease in GSH levels and cell death induced by cystine starvation (Chen et al. 2017). In a recent study, methionine, an indispensable amino acid crucial for amino acid metabolism, methylation reactions, and redox balance, was found to have a dual role in ferroptosis regulation (Xue et al. 2023b). *CHAC1* synthesis relies on methionine, and prolonged methionine deficiency reduces *CHAC1* production, hindering tumor cell ferroptosis. However, brief methionine depletion triggers ATF4 activation, subsequently stimulating *CHAC1* synthesis and increasing tumor cell susceptibility to ferroptosis (Xue et al. 2023b).

Sestrin 2 (*SESN2*) is known for its role in protecting cells against stress and maintaining cellular homeostasis (Kim et al. 2015). However, in dendritic cells, a surprising observation was made regarding *SESN2*'s interaction with the ATF4-DDIT3-*CHAC1* signaling axis, which seemed to enhance ferroptosis induction (Wang et al. 2020). When dendritic cells are exposed to lipopolysaccharide treatment, the ATF4-DDIT3-*CHAC1* pathway becomes more pronounced. This effect is further amplified upon *SESN2* knockdown, but can be alleviated by using an ATF4 inhibitor. These findings shed light on the crucial role of the *SESN2*-ATF4-DDIT3-*CHAC1* signaling axis in shaping dendritic cell responses to inflammation-induced ferroptosis under septic conditions (Li et al. 2021b; Wang et al. 2022c).

Studies have uncovered that cadmium induces ferroptosis in renal tubular epithelial cells through the EIF2AK3-ATF4-DDIT3 signaling pathway, especially under conditions of ER stress (Zhao et al. 2021). Effective mitigation of cadmium-induced ferroptosis can be achieved by inhibiting ER stress (Zhao et al. 2021). Similarly, in human umbilical vein endothelial cells, the activation of ER stress activates the HSPA5-ATF4-DDIT3 axis, where active DDIT3 downregulates GPX4, thereby promoting ferroptosis (Zhu et al. 2023). On the other hand, ATF4-mediated DDIT3 activation not only modulates autophagy, but also regulates the expression of various autophagy-related genes (B'Chir et al. 2013). In hepatocytes, the upregulation of DDIT3 fosters excessive autophagy, including ferritinophagy, leading to the degradation of ferritin heavy chain 1 (FTH1), an increase in iron ion levels, and the promotion of ferroptosis (He et al. 2022). This also illustrates a positive feedback loop mechanism in ATF4-DDIT3-induced ferroptosis, particularly evident in cases where iron overload triggers ferroptosis in pancreatic beta cells through the activation of the MAP3K5-p38-DDIT3 pathway (Deng et al. 2023). The mechanism by which ATF4-dependent DDIT3 selectively mediates ferroptosis or apoptosis in response to cancer therapy remains unclear (Ludwig et al. 2023).

Another player in the context of ATF4-related ferroptosis is BTG1, a member of the ErbB2 family of BTG/

transposons. Originally discovered alongside MYC in B-cell chronic lymphocytic leukemia (Rimokh et al. 1991). BTG1 plays a role in influencing cell cycle progression, differentiation, and cell death through its interaction with ATF4 (Yuniati et al. 2016). In hepatocytes undergoing ferroptosis, ATF4 positively regulates the expression of BTG1. This heightened BTG1 expression, in turn, augments ATF4 activation, including the upregulation of ATF4-dependent *TRIB3* and *DDIT3* genes (Cho et al. 2021). Furthermore, *SLC1A5*, a target gene of ATF4, exhibits reduced glutamine uptake and decreased MDA accumulation in melanoma cells when miR-137 is inhibited, resulting in suppressed ferroptosis (Luo et al. 2018). Nevertheless, the exact mechanisms of the ATF4-*SLC1A5* axis in influencing ferroptosis remain to be fully elucidated.

In summary, ATF4 plays dual roles in ferroptosis, but the circumstances determining whether ATF4 acts as an inhibitor or agonist of ferroptosis remain unclear. A deeper understanding of ATF4's regulatory role in ferroptosis may offer novel disease treatment approaches and insights into ferroptosis mechanisms.

ATF4 in cancer

Ferroptosis plays a dual role in tumor biology, with potential as a direct cancer cell eradication strategy, yet contributing to tumor development and immune response attenuation (Chen et al. 2023, 2021b; Dai et al. 2020b; Kim et al. 2022a; Motooka and Toyokuni 2023). ATF4 also exhibits a complex role in tumorigenesis and tumor therapy, dependent on tumor stage and microenvironment (Fig. 4) (He et al. 2023; Horiguchi et al. 2012; Xiao et al. 2019). ATF4's multifaceted role in tumors requires careful evaluation across different contexts, with the following sections elaborating on its dual roles and potential as a therapeutic target in tumor therapy.

ATF4 acts as an oncogene

In the tumor microenvironment, hypoxia is a prevailing condition where cancer cells adeptly respond to their surroundings (Masson and Ratcliffe 2014). Hypoxia induces the rapid upregulation of lactate dehydrogenase A (LDHA) through hypoxia-inducible factor 1 subunit alpha (HIF1A) (Semenza 2013). This enzyme aids in converting pyruvate to lactate, compensating for compromised oxidative mitochondrial function during hypoxia, and thereby sustaining tumor cell survival. Conversely, in melanoma cells, inhibiting LDHA triggers a signaling cascade involving EIF2AK4-ATF4 activation, driving serine and aspartate biosynthesis, upregulating *SLC1A5*, and bolstering glutamine and essential amino acid influx (Pathria et al. 2018). This pathway ultimately

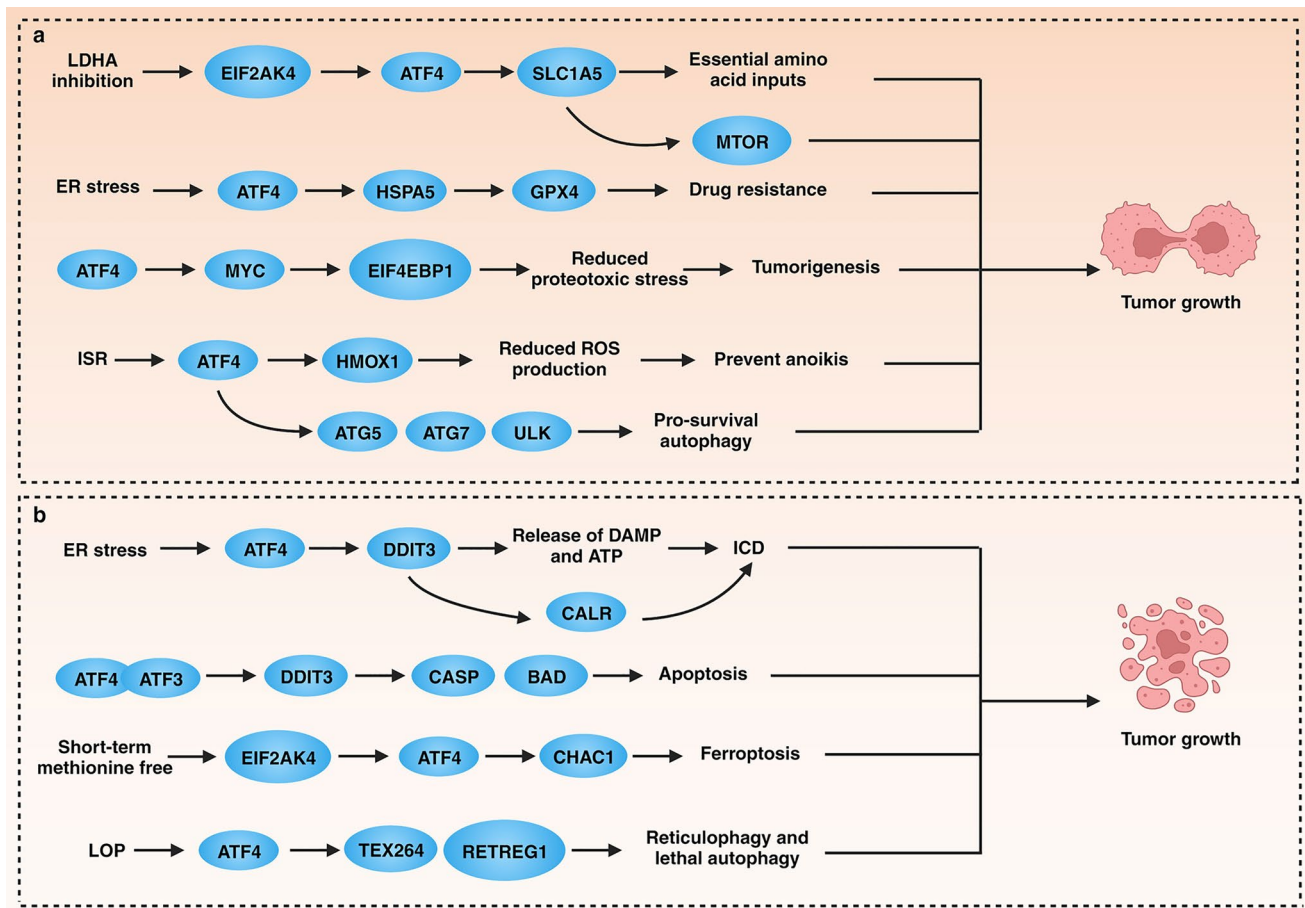


Fig. 4 The role of ATF4 in cancer. ATF4 governs the expression of distinct target genes in various contexts, thereby shaping the destiny of tumor cells. **a** ATF4 acts as a tumor-promoting factor by enhancing

survival mechanisms, including cellular resistance to drugs and evasion of cell death. **b** ATF4 also acts as a tumor suppressor, inducing cell death and strengthening immune responses

contributes to pro-survival MTOR activation (Pathria et al. 2018).

In addition, tumor cells often experience disrupted protein folding in the ER (Wang and Kaufman 2014). In PDAC cells, erastin induces ER stress, triggering the ATF4-HSPA5-GPX4 pathway, which confers resistance to gemcitabine by inhibiting ferroptosis (Zhu et al. 2017). Furthermore, ATF4 contributes to tumorigenesis by binding to over 30 promoter regions of MYC proto-oncogene (MYC) target genes, particularly those involved in amino acid and protein synthesis (Dey et al. 2013; Qing et al. 2012). This interaction involves eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1), which mitigates MYC-induced proteotoxic stress (Tameire et al. 2019). MTOR negatively regulates EIF4EBP1, and blocking MTOR activity in *ATF4*-deficient cells counteracts MYC-induced ER stress, delaying MYC-driven tumorigenesis (Tameire et al. 2019). These findings establish a complex interplay between ATF4, proteotoxic stress, and proliferative signaling during tumorigenesis (Singleton and Harris 2012).

The migration and invasion of cancer cells require detachment from the extracellular matrix (ECM), enabling their entry into blood and lymphatic vessels (Yilmaz and Christofori 2009). When HT1080 cells detach from the ECM, they activate the ISR (Dey et al. 2015). This triggers ATF4-mediated signaling, serving a dual purpose. First, ATF4 promotes the upregulation of the antioxidative stress factor heme oxygenase 1 (HMOX1), which reduces ROS generation and guards against anoikis—a form of cell death resulting from the loss of ECM attachment (Dey et al. 2015). Second, ATF4 stimulates pro-survival autophagy by increasing the expression of autophagy-related genes, including ATG5, ATG7, and unc-51 like autophagy activating kinase (ULK) (Dey et al. 2015).

In summary, ATF4's oncogenic role in tumor cells often arises due to its upregulation, leading to enhanced cell survival, migration, and invasion in response to stress conditions (Fig. 4a).

ATF4 acts as a tumor suppressor

ATF4 also can suppress tumorigenesis (Fig. 4b). Immunogenic cell death (ICD) is a mode of cell demise characterized by the release of damage-associated molecular patterns (DAMPs) such as HMGB1 and ATP, along with the presentation of calreticulin (CALR) on the cell surface. These processes collectively promote dendritic cell maturation and activation, ultimately leading to antigen presentation and cytotoxic T-cell responses (Galluzzi et al. 2020; Martins et al. 2014; Tang et al. 2023a). ATF4 can enhance anti-tumor activity by promoting antitumor immune responses, including ICD. For instance, oleandrin, a cardiac glycoside, exemplifies this process in breast cancer cells through ER stress-induced activation of EIF2AK3-ATF4-DDIT3 signaling, independent of the CASP family (Li et al. 2021d). In this context, ER stress increases DAMP and ATP secretion, intensifying immune cell infiltration and response (Li et al. 2021d).

Gene-level regulation also plays a role in ATF4-mediated tumor suppression. For example, withaferin A-induced cell death in glioblastoma multiforme triggers ATF4-ATF3-DDIT3 activation, promoting apoptosis and cell cycle arrest through transcriptional regulation (Tang et al. 2020). The ablation of ATF4 in hepatocytes inhibits hepatic steatosis but increases susceptibility to ferroptosis through the downregulation of SLC7A11, consequently resulting in the accelerated development of hepatocellular carcinoma (He et al. 2023). Additionally, a brief deficiency in methionine can activate the ATF4-CHAC1 pathway, increasing the susceptibility of tumor cells to ferroptosis (Xue et al. 2023b). Consequently, a combined approach involving short-term dietary methionine deficiency, a ferroptosis inducer, and an anti-programmed cell death 1 (PDCD1, also known as PD-1) antibody yields significant inhibition of tumor progression (Xue et al. 2023b). These findings also establish dietary approaches to enhance ferroptosis-related antitumor immunity.

The interplay between ATF4 and autophagy plays a role in determining the fate of tumor cells. In some situations, ATF4-induced autophagy can promote cell survival by eliminating damaged components and providing energy during stressful conditions. For instance, in colon cancer cells, suppression of glutamine catabolism activates the ATF4-DDIT4 signaling pathway, leading to the inhibition of the MTOR and the initiation of pro-survival autophagy (Han et al. 2021b). In lung cancer cells, the survival process during glutamine starvation, facilitated by ATF4, is also positively regulated by the activation of the Hippo pathway (Kim et al. 2023). However, under specific circumstances, excessive or sustained ATF4 activation and autophagy can lead to a form of cell death known as "autophagy-dependent cell death" (Chen et al. 2023; Denton and Kumar 2019; Li et al. 2021c; Liu et al. 2023; Tang et al. 2019; Xie et al. 2023a), which

functions as a tumor suppressor mechanism. The precise conditions or stimuli that determine whether ATF4 promotes cell survival through autophagy or contributes to cell death through excessive autophagy are not yet fully understood.

Conclusion and perspective

In summary, ATF4 plays a pivotal role in cellular stress responses, ferroptosis, and cancer, exhibiting its versatility in adapting to adverse conditions, such as ER stress, amino acid deprivation, and oxidative stress (Dey et al. 2015; Wang et al. 2022b, 2023). Its intricate involvement in the ISR influences cell survival and shapes cancer biology.

The emergence of ferroptosis as a cell death pathway in cancer therapy highlights ATF4's contextual role, either promoting or inhibiting ferroptosis (Chen et al. 2017; Zhu et al. 2017). This dual function suggests that ATF4-mediated mechanisms can offer resistance to ferroptosis, but may also represent therapeutic targets.

For future directions, deeper insights into ATF4's multifunctionality, interactions with other cellular components, and context-dependent actions are essential for innovative cancer treatments. Leveraging ATF4 as a modulatory target, whether to enhance ferroptosis in apoptosis-resistant cancers or counteract tumor-promoting effects, presents promising therapeutic avenues. Additionally, uncovering the molecular intricacies of ATF4's role in cell stress and ferroptosis may lead to biomarker discovery and predictive indicators in diverse cancer types.

In conclusion, the dynamic interplay among ATF4, cellular stress, ferroptosis, and cancer underscores the complexity of cellular systems. It emphasizes the need for a comprehensive approach considering the intricate cross-talk between stress pathways and their implications in cancer progression. As research reveals the molecular nuances of these interactions, novel targeted therapies exploiting ATF4's dual nature in stress response and cell death regulation may reshape the landscape of cancer treatment.

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Data availability All data supporting the findings of this study are available within the paper.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed.

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