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Regulation of Cellular Immunity by Activating Transcription Factor 4

Debasmita Mukherjee[1], **Lena S. Bercz**[1], **Molly A. Torok**[1], **Thomas A. Mace, PhD**[1,2]

[1]The James Comprehensive Cancer Center, The Ohio State University, Columbus, OH

[2]Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition; The Ohio State University, Columbus, OH

Abstract

Activating transcription factor 4 (ATF4) is a DNA binding transcription factor belonging to the family of basic Leucine zipper proteins. ATF4 can be activated in response to multiple cellular stress signals including endoplasmic reticulum stress in the event of improper protein folding or oxidative stress because of mitochondrial dysfunction as well as hypoxia. There are multiple downstream targets of ATF4 that can coordinate the regulation between survival and apoptosis of a cell based on time and exposure to stress. ATF4, therefore, has a broad range of control that results in the modulation of immune cells of the innate and adaptive responses leading to regulation of the cellular immunity. Studies provide evidence that ATF4 can regulate immune cells such as macrophages, T cells, B cells, NK cells and dendritic cells contributing to progression of disease. Immune cells can be exposed to stressed environment in the event of a pathogen attack, infection, inflammation, or in the tumor microenvironment leading to increased ATF4 activity to regulate these responses. ATF4 can further control differentiation and maturation of different immune cell types becoming a determinant of effective immune regulation. Additionally, ATF4 has been heavily implicated in rendering effector immune cells dysfunctional that are used to target tumorigenesis. Therefore, there is a need to evaluate where the literature stands in understanding the overall role of ATF4 in regulating cellular immunity to identify therapeutic targets and generalized mechanisms for different disease progressions.

Keywords

ATF4; Unfolded protein response; ER stress; Cellular Immunity

Address correspondence to: Thomas A. Mace, Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition, The Ohio State University, 420 W 12th Ave., Columbus, OH 43210 (thomas.mace@osumc.edu). Tel:614-366-3247.

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1. Introduction

Activating transcription factor 4 (ATF4) belongs to the basic leucine zipper (bZIP) transcription factor superfamily and is a master regulator that plays a crucial role in the adaptation to stresses.[1] ATF4 participates in the integrated stress response (ISR), involved in amino acid metabolism, differentiation, metastasis, angiogenesis, resistance to oxidative stress and drug resistance. Several ATF4 downstream target genes are themselves transcription factors that regulate the expression of a set of stress-induced target genes and amplify the signal initiated by the original stress. [2]

1.1 ATF4 regulates cellular stress

ATF4 mostly responds to immune cells via the endoplasmic reticulum (ER) stress mediated Unfolded Protein Response (UPR) pathway (Figure 1). The endoplasmic reticulum is the organelle responsible for biosynthesis, folding and modification of membrane bound as well as secretory proteins. Different physiological demands might contribute to improper folding of the proteins leading to the accumulation of unfolded or misfolded proteins in the ER lumen, leading to ER stress and is tackled by the UPR. UPR has two distinct phases–the adaptive phase where the response reduces the burden of accumulated proteins via providing chaperones to fold proteins properly or by reducing overall production of the proteins. The translational attenuation is classically attributed to the phosphorylation of the translational initiation factor eIF2α and recently identifies secondary regulation involving a downstream ISR target, 4E-BP, in the inhibition of eIF4E and specifically cap-dependent translation. [3] Misfolded proteins unable to be fold properly via chaperone proteins expressed through ISR result in terminally misfolded proteins which are either targeted for proteasomal degradation or ER associated degradation (ERAD). The major ISR effector molecule that is produced as a result of eIF2α phosphorylation and uORF translation is ATF4. ATF4 can also direct the cell to autophagy. However, if the stress continues to persist, the UPR switches to its apoptotic phase to promote cell death. [4–7] Three ER-resident transmembrane proteins orchestrate the UPR: the protein kinase R (PKR)-like ER kinase (PERK), activating transcription factor-6 (ATF6), and inositol requiring enzyme-1 alpha (IRE1a). In unstressed cells, these proteins are inactive because their luminal domains are associated with Binding Immunoglobulin protein (BiP) / 78 KDa Glucose -regulated protein (GRP78). During stressed conditions, misfolded/unfolded proteins compete and are preferentially bound to GRP78, disassociating PERK, ATF6 and IRE1, thereby activating them in the process. [8] IRE1 is a transmembrane Ser/Thr kinase with additional endonuclease activity. Mammalian IRE1 occurs as two homologues of the yeast genome, IRE1a and IRE1b, with similar but non-identical cleavage specificities in a temporal as well as tissue specific manner. In presence of unfolded proteins, IRE1a auto-phosphorylates to become active as a ribonuclease and targets a bZIP transcription factor X-Box DNA Binding Protein 1 (XBP-1). XBP-1 mRNA is therefore cleaved by IRE1a with the removal of a 26-nucleotide intron introducing a translational frameshift that forms the XBP-1 isoform (XBP-1s) with a novel carboxy terminus. XBP-1s acts as a potent transcription activator that can produce various ER chaperone proteins restoring ER homeostasis and further upregulating the expression of GRP78. [9–12] Upon ER stress induced disassociation with GRP78, two Golgi localization signals within the ER luminal domain of ATF6a (one of the two homologues of mammalian

ATF6) get exposed translocating it to the Golgi apparatus. ATF6a is then cleaved by site1 protease and site2 protease sequentially removing the luminal domain and transmembrane anchor, respectively, resulting in a 50 KDa amino-terminal cytoplasmic fragment that can enter the nucleus and bind to the ER stress response elements (ERSE). [8, 13] ATF6a, like XBP-1s, can upregulate the expression of GRP78 by binding through ERSE in the promoter region of GRP78. ATF6a can further induce the expression of other UPR mediators like XBP-1 providing more substrate for IRE1a along with chaperone proteins. [14, 15] Therefore, a positive feedback loop between GRP78, ATF6 AND XBP-1 is established.

Upon dissociation of PERK with GRP78, PERK is activated which can thereafter phosphorylate eIF2a. Phosphorylation of eIF2a reduces ER client protein load by inhibiting global protein synthesis but allowing selective mRNAs such as ATF4 to be preferentially translated. eIF2a phosphorylation resulting in upregulated expression of ATF4 can also be catalysed via general control non-derepressible 2 (GCN2) because of amino acid limitation and UV exposure or via protein kinase RNA-activated (PKR) activated in response to viral infections or via heme-regulated inhibitor (HRI) activated by heme deprivation and oxidative stress. [16] Depending on context, ATF4 can control expression of genes involved in amino acid transport and metabolism, protection from oxidative stress, and protein homeostasis. ATF4 can also push the cell towards autophagy through various processes such as upregulating the expression of a protein called REDD1 that inhibits mTORC1 in a TSC1/TSC2-dependent manner [17, 18] and can induce apoptosis, cell-cycle arrest, and senescence keeping a fine balance between life and death of a cell. [5, 19]

1.2 ATF4 mediates CHOP activity

ATF4, in conjunction with ATF6, transactivates the transcription factor C/EBP homologous protein (CHOP). CHOP was also found to regulate mediators of apoptosis such as B-cell lymphoma 2 (BCL2) and BCL2 interacting mediator of cell death (BIM). [20, 21] Increased expression of CHOP has been reported to lead to upregulation of pro-apoptotic BH3 domain-only proteins genes such as BIM and downregulation of the anti-apoptotic protein Bcl-2 genes, along with disruption of the redox homeostasis. [22] CHOP has further been reported to induce the expression of Tribbles 3 (TRB3), which is a negative regulator of the survival pathway mediated by AKT.[23, 24] Under prolonged stress, CHOP and ATF4 have been reported to dephosphorylate eIF2a by activating Growth arrest and DNA damage-inducible protein (GADD34) increasing the nascent protein load promoting more ER stress and increasing apoptotic cell death. [25] Further, CHOP can induce expression of ER oxidoreductin 1α genes that promote a hyper-oxidising environment through generation of reactive oxygen species (ROS) and lead to apoptosis. Generation of ROS with increased protein synthesis before restoring protein homeostasis marks the apoptotic phase of UPR. [26] However, multiple reports of ATF4 inducing apoptosis in a CHOP independent manner with unexpected increase in cell death after CHOP knockdown has suggested pro-survival functions of CHOP. [27, 28] Therefore, the transcriptional regulation of CHOP via ATF4 is a complex phenomenon.

1.3 Hypoxic stress activates ATF4

Hypoxic stress results in a rapid and sustained inhibition of protein synthesis that is at least partially mediated by eIF2α phosphorylation by the PERK and therefore can also trigger the activation of ATF4. [29, 30] ATF4 is a major transcriptional regulator of the cellular hypoxic response apart from Hypoxia-inducible factor 1α (HIF1α) and is responsible for the activation of genes that provide favourable conditions for normal ER function and promote survival.[6, 31–33] Recently, the 154–181 amino acid region of ATF-4 was identified to interact with Prolyl-4-hydroxylase domain (PHD), which is hydroxylated in an oxygen dependent manner and forms the molecular basis for the hypoxia-induced stability and activity of HIF-1α and HIF-2α. The study found upregulation of ATF-4 protein after treatment with hypoxia or the PHD inhibitor Dimethyloxallyl Glycine (DMOG), demonstrating that mechanisms other than translational control might be additionally involved in the regulation of ATF-4 protein stability. [34]

This has led to a lot of interest in understanding the role of ATF4 in the tumor microenvironment, as it has been seen that cancer cells can survive under hypoxic and metabolic stress. A study reported that translation reprogramming via microenvironmental stress signals through UPR drives phenotypic plasticity and invasion, determining therapeutic outcomes in melanoma using cell-based experiments as well as transcriptomic analysis of data from human melanoma patients from The Cancer Genome Atlas (TCGA) data sets. Translational reprogramming was confirmed by observing tumor colonization in the lungs using B16 melanoma mice model. [35] In addition to the UPR in tumor cells being studied as a cell-intrinsic mechanism for cell survival, the ER stress response has been shown to aid tumor growth in a cell-extrinsic manner by inhibiting antitumor immunity via T cell-independent and -dependent mechanisms. [36] A study investigating ovarian cancer reported that the ER stress response in the tumor microenvironment can result in ROS-dependent activation of PERK in the induction of CHOP in tumor-exposed T cells regulating their antitumor activity. [37] However, the overall role of ATF4 in regulating the activity of immune cells through response towards mitigation of ER stress or as a result of inflammatory response has not been extensively reviewed. In this review, the role of ATF4 in regulating the overall cellular immunity as well as immune cell maturation, polarization state, and responses in progression of diseases will be explored (Table 1).

2. T cells

T cells are one of the major components of the adaptive immune system that can recognize a specific antigen leading to activation from a naïve T cell to an activated phenotype with effector functions. They can mature to form $CD8^+$ T cells, with the ability to recognize and kill target cells expressing the antigens presented by Major Histocompatibility Complex (MHC) class I, leading to the release of cytotoxic molecules such as perforin and granzymes. $CD4^+$ T cells or helper T cells have a wide range of activity including shaping and regulating other adaptive immune responses. $CD4^+$T cells can differentiate into several different subtypes of T helper (Th) cells such as Th1, Th2, Th9, Th17, and T regulatory cells, maintaining a tighter immune regulation. Memory T cells are a small subset of cells

that remain in the body following initial exposure to a specific antigen. Memory T cells are important in quick expansion of effector T cells on re-exposure to the same antigen. [38]

T cell proliferation and function is regulated by a multitude of factors, including the availability of extracellular amino acids and the oxidizing environment. Arginine depletion has been reported to affect T cell proliferation. [39] Additionally, activated T cells have been shown to have higher metabolism of L-arginine, resulting in enhanced $CD4^+$ and $CD8^+$ T cell survival. [40] Another novel finding was reported on the critical role of serine metabolism in activated T cells supporting T cell proliferation. Serine and glycine-limiting conditions can impair anti-CD3/CD28 antibody-driven $CD4^+$ and $CD8^+$ T cell proliferation *in vitro*, antigen-driven $CD8^+$ effector T cell expansion, and pathogen clearance in mice. [41] ATF4 has been reported to be induced by the extracellular oxidizing environment which can therefore target a network of genes encoding proteins that control the metabolic flux. Enhanced anabolism leads to an overall increase of amino acid and protein synthesis, helping T cells to proliferate under stress. [42] ATF4 has been further shown to contribute to T cell growth in oxygen or amino acid limited environment by participating in catabolism via upregulating genes required in the glycolysis pathway, as well as promoting anaplerotic flux by enhancing glutaminolysis. [43] ATF4 deficiency reportedly results in reduced Th1 and increased Th17 responses *in vivo* with a reduction of Th1 differentiation and an increase in factors driving Th17 phenotype (characterized by increase in the expression of InterLeukin - 17 (IL-17) in myeloid cells.[43, 44] This is further validated by investigating the small molecule halofuginone (HF) which can inhibit Th17 differentiation in mice and humans. The addition of excess amino acids rescues the inhibition of Th17 differentiation by HF suggesting Th17 differentiation is targeted by activating the amino acid starvation response (AAR), implicating the AAR pathway in regulation of inflammatory T cell differentiation *in vivo*. [45] As a result of amino acid deprivation or functional inhibition of L-type amino acid transporter 1 (also known as SLC7A5), ATF4 can further induce the expression of Homeobox B9 (HOXB9) in $CD4^+$ T cells activated in the presence of anti-CD3/CD28. HOXB9 can interfere with the activities of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), nuclear factor of activated T-cells (NFAT) and Activator Protein 1 (AP-1), but not the retinoic acid receptor-related orphan receptor. This combination results in attenuation of the production of selective cytokines in activated T cells such as interferon-gamma (IFNγ), IL-2 and IL-4 whereas IL-17 does not significantly change. [46] T cell regulation via ATF4 can therefore be a direct or indirect result of stress and can span different disease progression pathways.

Diseases caused by viral infections have also been shown to involve T cell responses regulated by ATF4. Most viral pathogens need to subvert the innate antiviral response in order to enter the host and replicate. *In vitro* and *in vivo* studies using human $CD4^+$ T cell culture models testing a simian immunodeficiency virus (SIV) model of AIDS were used to investigate how human immunodeficiency virus (HIV) can evade early innate defenses. Suppression of protein synthesis and induction of protein kinase GCN2-ATF4 signaling were detected in the gut during acute SIV infection that diminished during chronic viral infection. HIV replication induced in $CD4^+$ T cells reached a similar fate with the induction of ATF4 that was recruited to the HIV long terminal repeat (LTR) to promote viral transcription. Inhibition and enhancing ATF4 suppressed HIV expression and reactivated

latent HIV, respectively. [47] Similarly, the link between ER stress mediated apoptosis following a bystander HIV Tat stimulus was studied on Jurkat T cells. The stimulus resulted in time-dependent overexpression of major UPR markers including ER chaperone and stress sensors, as well as an increase in levels of downstream mediators including eIF2α, ATF4, XBP-1. Proapoptotic factors such as CHOP, GADD34, and BIM were also seen to increase. [48] These results provide a mechanism for the continuous depletion of uninfected CD4+ T lymphocytes observed in HIV-related disease. However, a study investigating LTR activation mediated by Tax concerning Human T-cell leukaemia virus type 1 (HTLV-1) showed all three members of the TORC family of transcriptional regulators as coactivators of Tax for LTR-driven expression, but not ATF4 or other bZIP factors. [49, 50] Therefore, the complex process of viruses to evade immune detection may or may not involve T cell regulation by ATF4.

Evasion of immune system detection by rendering T cells ineffective via ATF4 is also observed during cancer progression and becomes extremely important in the context of effective cancer immunotherapy. The tumor microenvironment can introduce distinct but interconvertible cell types that sustain malignant and therapy resistant phenotype including resistance to anti-PD1 immunotherapy. To understand the mechanisms that help different cancer cells invade and become drug resistant, a study was performed on transcriptionally repressed melanoma identified microphthalmia-associated transcription factor (MITF) by ATF4 in response to translation initiation factor eIF2B. ATF4 further activates AXL Receptor Tyrosine Kinase (AXL) and suppresses senescence to impose the MITF-low/AXL-high drug-resistant phenotype observed in human tumors. However, the ATF4-high/MITF-low state is insufficient to drive invasion without translational reprogramming, suggesting that there are microenvironmental stress signals which drive phenotypic plasticity, invasion and therapeutic outcome by translational reprogramming. [35] Another group investigating the mechanism behind dysfunctionality of CD8+ T cells in cancer immunotherapy reported that CHOP expression is increased in tumor-infiltrating $CD8^+$ T cells and correlates with poor clinical outcome in ovarian cancer patients. [37] CHOP is elevated by ATF4 as a result of persistent ER stress and directly represses Tbet expression. Thus, CHOP acts as a major negative regulator of the effector function of tumor-reactive $CD8^+$ T cells. The authors provide evidence that deletion of CHOP in T cells improves antitumor $CD8^+$ T cell immunity and therefore boosts the efficacy of T cell-based immunotherapy. This study suggests there is a therapeutic potential of blocking CHOP or ER stress to unleash T cell-mediated antitumor immunity.

ATF4 can also be targeted by different cancer therapeutic agents that involves regulation of different T cell responses. While studying the effect of farnesol on T lymphoblastic leukemic Molt4 cells, farnesol was found to activate the apoptosome. Gene expression analysis via microarray revealed the specific induction of the PERK-eIF2α-ATF3/4 cascade in a manner that is independent of the farnesol-induced activation of Mitogen-activated protein kinases (MAPKs). [51] Another study focused on understanding the role of sorafenib in targeting acute myeloid leukemia (AML) with an internal tandem duplication (ITD) in the gene encoding Fms-related tyrosine kinase 3 (FLT3). Sorafenib is a multi-targeted tyrosine kinase inhibitor and was shown to increase IL-15 production in FLT3-ITD^+ leukemia cells, which synergized with the allogeneic $CD8^+$ T cell response. Sorafenib-related IL-15

production caused metabolic reprogramming of leukemia-reactive T cells in humans via reduced expression of ATF4, thereby blocking negative regulation of interferon regulatory factor-7 (IRF-7) activation. [52] In other disease conditions, ER stress has been shown to result in the development of Crohn's disease-like ileitis mediated by cytotoxic CD8αβ^{+} intraepithelial lymphocytes (IELs). Heterogeneous knockout of Tumor Necrosis Factor (TNF$^{\Delta ARE/+}$) mice under chronic inflammation exhibited increased expression of ATF4 in addition to GRP78, ATF6, and spliced XBP1 in CD8αβ^{+} IEL but not in CD8αα^{+} IEL or in lamina propria lymphocytes. [53] In another study, a group examined the role of epigenetic modification of H3K9ac in regulation of CD4+ T lymphocytes in driving acute-on-chronic liver failure (ACLF) that displays 'sepsis-like' immune paralysis. In a study conducted by Jin et al., it was discovered that downstream pathway-related genes of ER stress response such as Heat Shock Protein Family A (Hsp70) Member 5 (HSPA5), BCL2 interacting mediator of cell death (BNIP1), IRF3, Apoliprotein-A4 (APOA4), PolyUbiquitin-C Precursor (UBC) and Period Circadian Regulator 1 (PER1) as well as ATF4, were differentially modified through utilization of ChIP microarray based functional analyses in ACLF. [54] Therefore, ATF4 can interact with T cells to maintain proliferation and differentiation, ensuring proper activity of different kinds of T cells as well as hijacking normal functioning to cause diseases such as viral infections and cancer.

3. Macropahges

Macrophages are derived from blood monocytes and are involved in the detection and phagocytosis of pathogens, antigen presentation to T cells, and initiating inflammation through the release of various cytokines. Macrophages are a heterogeneous set of cells that differentiate and reside in several different tissue locations based on their surrounding microenvironment. [55] Macrophages can respond to environmental cues and are able to change their function by transitioning between different polarization states. Such plasticity renders them the ability to secrete a range of various cytokines that are either pro-inflammatory or anti-inflammatory. [56] Environmental cues received in the form of cellular stress, such as hypoxia or ER stress, generated due to UPR, has been investigated. Mechanistically, ER stress signals have often been found to trigger the ATF4 pathway and interact with multiple factors related to macrophages to alter their plasticity. GRP78 can regulate macrophage function and insulin resistance via ATF4 in diet induced obesity. In GRP78 deficient mice, high levels of ATF4 mRNA lead to the activation of adaptive UPR, resulting in increased IL-6 expression in macrophages that polarizes them to the M2 state. This leads to stimulation of IL-13 signaling via upregulating IL-13 Receptor α1 (IL-13Rα1) and increasing glucose metabolism in skeletal muscle, making macrophage UPR signaling a potential pharmacological therapeutic target for treating skeletal muscle insulin resistance. [57]

Recent reports investigating the tumor microenvironment have implicated the importance of macrophage infiltration for sustenance of tumor cell survival and metastases. Expression of ATF4 in tumors of clinical samples correlated with macrophage infiltration. Studies show that ATF4- knockdown in endometrial cancer cells results in a reduction in M2 macrophage infiltration in xenograft animal models. Upon investigation, the tumor microenvironment related stress was observed to upregulate ATF4 expression, promoting the expression of

chemokine CCL2. This resulted in recruitment of macrophages, and therefore contributed to endometrial tumor growth. Thus, ATF4/CCL2 was shown to be a potential therapeutic target for tumor microenvironment. [58] Furthermore, ATF4 activity in breast cancer cells recruits macrophages via secretion of M-CSF. [59] Another intricate regulatory role of ATF4 in macrophages is emphasized by the down-regulation of ATF4 to suppress the M2 polarization phenotype for Retinoic acid-inducible gene (RIG) like receptor- based infection of West Nile Virus via the shift to the M1 phenotype. [60]

Hypoxia leads to up-regulation of certain genes to help respond to the stress caused by a lack of oxygen, out of which HIF-1α is a well-known central mediator. However, recent studies have suggested the induction of ATF4 as a pivotal transcriptional regulator to this stress since hypoxia is a frequent consequence to the UPR following ER stress. While investigating the regulation of macrophages in the ischemic areas of diseased tissue, ATF4 was found to be the hypoxia responsive factor in macrophages early after exposure. [61] Another study investigated Infantile Hemangioma (IH), a condition in which a mark or coloured patch appears within a few weeks after birth as a result of incorrectly formed blood vessels that multiply more than usual. A significant upregulation of ATF4 in proliferating IH was found in comparison to the involuting phase (typically in a year after proliferation and plateau phase). ATF4 was positively correlated with HIF-1α expression in IH specimens using the Spearman correlation and was further corroborated by their synchronous distribution through double labelled immunofluorescence. M2 macrophage polarization closely correlated to ATF4 expression, underlining a positive regulation mechanism of hypoxia-stimulated ATF4, leading to M-CSF based recruitment of M2 polarized macrophages. [62] Following the same route, the role of hypoxia in the induction of M2-polarized macrophage infiltration through ATF4 and its potential relationships with angiogenesis in odontogenic keratocysts (OKC) was also investigated. The pathway resulted in stimulation of the receptor activator of nuclear factor κ-B ligand (RANKL), leading to the development of OKC. This occurred as a result of elevated ATF4 expression in the epithelial lining of OKC in response to hypoxia. [63] Apart from regulating the survival function on exposure to metabolic stress, ATF4 can mediate apoptosis and hence is implicated in autophagy. Arthero-sclerosis is a condition of the cardiovascular tissue suffering from non-resolvable inflammation associating autophagy and formation of macrophage foam cells to contribute towards this dysfunction. While studying the role of cysteine proteases cathepsins in autophagy, cathepsin inhibition led to a mitochondrial stress and ROS production that triggered the ATF4-CHOP pathway. Upon transcriptomic analysis, these cells were genetically similar to inflammatory macrophages. [64] Similar results were reached while studying C/EBPβ regulation in macrophage foam cell formation, driving arthero-sclerosis where they observed (siRNA)-mediated knockdown of C/EBPβ attenuated atherogenic lipid-mediated induction of proteins and genes implicated in macrophage mediated inflammation, ER stress (ATF4 and ATF6), and apoptosis (CHOP). [65] Therefore, ATF4 has an overall ability to affect macrophage polarization as well as the ability to induce various phenotypic changes in the macrophages in order to direct responses to support disease progression.

4. B Cells

B cells are also a part of the adaptive immune response and mediate immunity by producing antigen-specific immunoglobulins directed against invasive pathogens. [66] Dysregulation in proper development and maturation of B cells is implicated in different kinds of abnormalities, spanning from immunodeficiency and autoimmunity to haematological malignancies, some of which arise as a result of interaction with the ATF4 pathway. B cell receptor (BCR) expression is necessary for survival and development of B cells. BCR signaling has been found to trigger CHOP to promote BCR-mediated lytic replication of gamma herpes virus 68 (MHV68) by suppressing upstream Bip and ATF4 expression. BCR-mediated MHV68 lytic gene expression in CHOP knockout cells was rescued by knocking out Bip and this rescue was blocked by ectopic ATF4 expression. Further, ATF4 inhibited promoter activity of the MHV68 lytic switch transactivator replication and transcriptional activator (RTA) implicating a complex interconnectedness between BCR signaling and ER stress mediated UPR to regulate the gammaherpes virus infection cycle. [67] Similar interplay was also indicated in a study investigating anti-tumor activity of artesunate for the treatment of B cell lymphoma. Gene expression analysis identified ER stress and UPR as the most affected pathways with a distinct upregulation of markers ATF4 and CHOP in malignant cells vs. normal cells and artesunate treatment significantly suppressed overall cell metabolism as a treatment strategy. [68]

B cells go through a complicated maturation process and many of its developmental stages have malignant counterparts where the dominance of a particular sub-clone leads to development of leukemia or lymphoma. Mutations in the tumor suppressor B-cell Translocation Gene 1 (BTG1) have been known to cause Acute Lymphoblastic Leukemia and diffused large B cell lymphoma along with its lower expression levels correlating with poor clinical outcomes for many solid malignancies. In a study investigating the loss of Btg1, they found Btg1 renders a survival advantage to primary mouse embryonic fibroblasts (MEFs) under stress conditions. Under stress, BTG interacts with ATF4 to recruit protein arginine methyl transferase (PRMT1) which methylates ATF4 on arginine residue 239 modulating cellular adaptation positively. Loss of BTG1 shifts the balance and allows for cells to survive instead of targeting genes downstream of ATF4 that are pro apoptotic. [69] Further implications of the ATF4 pathway in haematological malignancies were discovered while investigating the single agent and synergistic combinatorial efficacy of first in class small molecule impridone ONC201. ONC201 induced caspase-dependent apoptosis that involved activation of the integrated stress response (ATF4/CHOP) pathway along with the inhibition of Akt phosphorylation, Foxo3a activation, downregulation of cyclin D1, Inhibitor of Apoptosis Protein (IAP), and B cell lymphoma (Bcl-2) family members in multiple different haematological malignancies. [70] Thus, specific targeting of ATF4 as a part of the UPR could lead to new therapeutic approaches for treating haematological malignancies.

5. NK Cells

Natural Killer (NK) cells are a part of the innate immune response known for defense against viral infections and tumors without any priming. NK cells can secrete cytokines such as IFN-γ and TNF-α that can enhance the immune response by interacting with other

immune cells. The Natural Killer Group 2D (NKG2D) receptor plays an important role in protecting the host from infections and cancer via recognition of cells expressing induced self-proteins acting as a primary activation signal for NK cells. [71] It can override inhibitory signals received by other NK cell receptors. [72] Since the ER stress elicits inflammatory response, UPR related proteins were hypothesized to induce surface expression of NKG2D ligands. [73] As a result, one of the ligands for NKG2D receptor, UL16 Binding Protein 1 (ULBP1), which is a cell surface glycoprotein related to MHC Class I molecules and functions as a stress induced ligand [74] was studied. A forward genetic screen study found that ATF4 is a critical protein involved in ULBP1 transcription and surface expression, and was therefore important for the induction of ULBP1, but not other NKG2DLs, showing a specificity for ER stress induced NKG2DL. The result was further confirmed by demonstrating that knockdown of ATF4 strongly decreased ULBP1 transcription. ATF4 was shown to have direct ULBP1 promotor binding sites that directly transactivates the ULBP1 promoter. [75] However, the study did not report on the interaction of the NKG2DL with NK cells. The functional response of NK cells as a result of upregulated expression of NKG2DL would provide more understanding of NK cell activity under ER stress. Another study investigated NK cell function in Type II diabetes patients and ER stress was found to be an important mediator. ER stress was induced *in vitro* in normal NK cells through tunicamycin treatment which resulted in a significant decrease in NKG2D expression. This was coupled with an increase in the markers of the UPR including XBP-1s, ATF4 and CHOP in the patient NK cells indicating that ER stress is activated in vivo through both PERK and IRE1 sensors implicating the UPR pathway as a potential mechanism. [76] Similarly, in a study investigating enteritis or inflammation in intestinal epithelial cells, *Xbp1* (downstream target of UPR) deletion in the epithelium ($Xbp1^{\Delta IEC}$) is shown to cause increased expression of (ULBP)-like transcript 1 and its human orthologue cytomegalovirus ULBP via CHOP, downstream target of ATF4. Increased numbers of intraepithelial NKG2D-expressing group 1 innate lymphoid cells (ILCs; NK cells or ILC1) were observed in $Xbp1^{\Delta IEC}$ cells, which when blocked, suppressed cytolysis against ER-stressed epithelial cells *in vitro* and spontaneous enteritis *in vivo*. Depletion of $NK1.1^{+}$ NK cells also significantly improved enteritis revealing innate immune sensing of ER stress in IECs as an important mechanism of intestinal inflammation. [77] Further, a study to investigate the role of NK cells in promoting insulin resistance in normal human liver cell line (HL-7702 cells) pre-treated with osteopontin (OPN) discovered that hyperactivation of JNK and subsequent decrease of tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) is responsible for impaired insulin signaling. This was reversed by inhibiting ER stress, since hepatic NK cells were able to induce obesity-induced hepatic ATF4 mediated ER stress. [78] Additional research into how ATF4 affects NK cell ligands as well as tissue specific responses to drive different conditions such as insulin resistance could open up new avenues of immune cell based therapeutics.

6. Dendritic cells

Dendritic cells (DCs) develop in different tissue types for antigen presentation and have been found to respond in a variety of manners to cellular stress. [79] It was discovered that DCs mount a specific integrated stress response during which ATF4 and the GADD34/Ppp1r15a,

a phosphatase 1 (PP1) cofactor, was expressed with an extensive dephosphorylation of the translation initiation factor eIF2α during DC activation. GADD34 was shown to be required for normal cytokine production both *in vitro* and *in vivo*, displaying the importance of pathogen detection with the integrated stress response pathways. [80] Similarly, in another study investigating responses to ER stress, different mucosal DCs were reported to respond in a tissue specific manner either via the ATF4- dependent cellular stress adaptation or via IRE1-dependent ER stress adaptive mechanism, a signaling pathway that also controls development and survival of immune cells. Lung circulating dendritic cells (cDC1s) die, whereas intestinal cDC1s survive via their ability to shut down protein synthesis through a protective integrated stress response by marked increase in regulated IRE1-dependent messenger RNA decay. [81] Furthermore, Sestrin2, a highly evolutionarily conserved protein, was reported to be expressed in dendritic cells after high mobility group box-1 protein stimulation to inhibit the apoptotic ER stress signaling based PERK-ATF4-CHOP mediated cell death pathway exerting a protective effect on DCs in the event of sepsis. [82] Further work in the field in understanding interactions of ATF4 with DCs will present a better understanding of the crossroads of antigen presentation, immune activation and cellular stress.

7. Myeloid-derived Suppressor Cells

On exposure to pathogenic stimuli, the innate immunity fights infection/inflammation via non-specific defenses. Pathogen associated molecular patterns (PAMPs) or danger associated molecular patterns (DAMPs) send strong signals to activate and expand neutrophils and monocytes followed by phagocytosis, respiratory burst and release of inflammatory cytokines. This innate response is short-lived and stops with the signal. However, persistent stimulation associated with chronic infection, inflammation, or cancer involves relatively low-strength signals that result in a different cell type with distinct genomic and biochemical properties through myelopoiesis. The main functional characteristic of these cells is their ability to suppress various types of immune responses and are therefore called myeloid derived suppressor cells (MDSCs). [83] They are of two types: granulocytic/ polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs). MDSCs mostly suppress immune activity by targeting T cells. M-MDSC suppress T cell responses both in antigen-specific and non-specific manners whereas PMN-MDSC primarily suppress T cell response in an antigen-specific manner. ATF4 can also interact with MDSCs to regulate their activity. [84–86] Chop has been reported in the accumulation and immune inhibitory activity of tumor-infiltrating MDSCs. Chop expression in MDSCs was shown to be driven by ATF4 in the presence of tumor-mediated reactive oxygen and nitrogen species. [87] In contrast, Chop-deficient MDSCs display reduced signaling through CCAAT/ enhancer-binding protein-β which leads to a reported decrease in the production of interleukin-6 (IL-6) and low expression of phospho-STAT3. This study further suggests Chop as a therapeutic target for cancer immunotherapy. [87] Another putative therapeutic target for immuno-oncology is GCN2, which is an environmental sensor in response to nutrient availability. Using mass cytometry as well as transcriptomics and transcription factor binding analyses, myeloid lineage deletion of GCN2 was shown to drive a shift in MDSCs and tumor associated macrophages promoting anti-tumor immunity. [88] Further

assessment on the mechanisms showed that GCN2 promotes translation of ATF4 and increases activation of pro-inflammatory MDSCs, macrophages and IFN-γ expression in intratumoral CD8+T cells adversely affecting the microenvironment. Thus, ATF4 can regulate overall MDSC activity in the tumor microenvironment.

8. Other inflammatory diseases

ATF4 can regulate survival, apoptosis and differentiation in immune cells and can therefore use them to drive tumorigenesis, autophagy and viral entry. However, the immune cell regulation via ATF4 is not limited to these kinds of disease progression and has been reported in broader ranges. Fungal keratitis driven by *Aspergillus fumigatus* (*A. fumigatus*) can damage visual acuity and cause blindness. [89–91] In a study investigating the response to the fungal infection, ATF4 was increased in corneas from two kinds of *A. fumigatus* keratitis models after 3 days as well as in the conidia in both the human corneal epithelial cells (HCECs) and the THP-1 macrophages 16 hours after stimulation. The ATF4 expression was shown to be dependent on Toll-like receptor 4 (TLR4), lectin-type oxidized LDL receptor 1 (LOX-1) expression, and MAPKs pathway and is therefore involved in the host antifungal immune response. [92] Arsenic has been shown to induce immunosuppression on chronic exposure. While investigating ATF4 in regulating arsenic trioxide (ATO)-mediated dysregulation of macrophage functions, ATO-treated ATF4(+/+) wild-type mice were compared to ATO-treated ATF4(+/−) heterozygous mice where the wild type mice showed a significant down-regulation of CD11b expression associated with the reduced phagocytic functions of peritoneal and lung macrophages. Further, ATF4 knockdown rescued ATO-mediated impairment of macrophage functions including cytokine production, bacterial engulfment, and clearance of engulfed bacteria in RAW 264.7 cells, suggesting that ATF4 plays an underlying role in pathogenesis of macrophage dysregulation and immune-toxicity of arsenic. [93] Another report showed that ATF4 is critical in the regulation of Monocyte chemoattractant protein 1 (MCP1) in retinal and brain microvascular endothelial cells. MCP1 is a chemokine that recruits monocytes to site of tissue injury and plays a role in microvascular complications of diabetes. In cases where Lipopolysaccharide (LPS) treatment was used to induce MCP-1, it was shown that overexpression of ATF4 enhanced retinal levels of MCP-1 and promoted inflammatory cell infiltration into the vitreous and retina, whereas LPS-induced MCP-1 upregulation was markedly attenuated in ATF4-deficient endothelial cells and in the retinas of ATF4 knockout mice. Furthermore, pharmacological inhibition of NF-κB, P38, or c-Jun N-terminal kinase JNK, significantly reduced the ATF4-stimulated MCP-1 secretion from endothelial cells. This suggests that regulation of MCP1 via ATF4 may contribute to inflammation-related endothelial injury in diseases such as diabetic retinopathy. [94] In another report investigating overcoming host defenses by the parasite *Leishmania* to cause *Leishmania amazonensis* infection, the PERK/eIF2α/ATF4 signaling branch of the integrated ER stress response was shown to be activated like many viral entry systems. Infected patient lesions showed increased expression of ATF4 and Heme oxygenase-1 (HO-1) mRNAs, whereas knocking down ATF4 in RAW 264.7 macrophage cells decreased nuclear factor erythroid 2-related factor 2 (NRF2) expression and its nuclear translocation, reducing HO-1 expression and increasing nitric oxide production. This suggests the importance of the ATF4 pathway in parasite survival and

progression, especially because human leishmaniasis infection is also associated with HIV infection. [95] A more general regulatory role of ATF4 as a part of the innate immune response was recently explored where expression of NACHT, LRR, FIIND, CARD domain and PYD domains-containing protein 1 (NLRP1), a core inflammasome component, is specifically up-regulated during severe ER stress conditions in human cell lines. Using mutagenesis, chromatin immunoprecipitation and Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9-mediated genome editing technology, the ATF4 transcription factor was shown to directly bind to the NLRP1 promoter during ER stress and regulate inflammation. [96] Therefore, the ability of ATF4 to regulate cell survival or lead to apoptosis in response to stress makes interactions with multiple stages of innate immune system homeostasis essential.

8. Conclusion

Activating Transcription Factor 4 is a stress induced transcription factor that can regulate a multitude of different immune cell responses. The regulation of immunity via ATF4 can be indirect by initiating inflammation that will secrete a cascade of cytokine directed immune responses. In contrast, ATF4 can also directly interfere with maturation, development, and polarization states of different immune cells, rendering tissue specific responses and therefore contributing to an overall modulation in immune cell regulation (Figure 2). ATF4 can be triggered by different stressors leading to its involvement in a range of different immune cell regulation processes. As tumor cells survive under extreme metabolic stresses, ATF4 is suggested to have an extensive role in dysfunctionality of different effector immune cells. Therefore, there is a huge potential in targeting ATF4 as an immunotherapeutic approach to cancer. However, more in-depth analyses of its involvement in tissue specific regulation of microenvironment is required in the field. ATF4 has also been reported to aid viral entry and evade the immune system by interacting with multiple immune cell types. A holistic approach in utilising ATF4 as a target for defense against viral entry can help explore different therapeutic potentials. The exposure to stress is time sensitive and the transition between the adaptive and apoptotic phase of the unfolded protein response is mediated by ATF4. Henceforth, many autophagy mediated disease progressions involve immune cell regulation via ATF4 and requires further research for a better understanding. This review has highlighted many investigations involving immune cell regulation by ATF4 and further mechanistic findings involving downstream signaling will provide new insights for the basic understanding of different immunological processes as well as to find previously unexplored therapeutic targets for different diseases.

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Abbreviations

AIDS	Acquired immune deficiency syndrome

AKT	Protein Kinase B
AML	Acute Myeloid Leukemia
APOA4	Apoliprotein A4
AP-1	Activator Protein 1
ATO	Arsenic Trioxide
ATF	Activating Transcription Factor
AXL	AXL Receptor Tyrosine Kinase
BCL2	B-cell lymphoma 2
BCR	B-Cell Receptor
BIP	Binding Immunoglobulin Protein
BIM	BCL2 interacting mediator of cell death
BNIP1	BCL2 interacting protein 1
BTG1	B-cell Translocation Gene 1
bZIP	basic leucine zipper
CCL2	Chemokine (C-C motif) ligand 2
cDC	Conventional Dendritic cell
CHOP	C/EBP homologous protein
CRISPR	Clustered regularly interspaced short palindromic repeats
DAMPs	Damage associated molecular patterns
DC	Dendritic cells
DDIT3	DNA damage-inducible transcript 3
DMOG	Dimethyloxallyl Glycine
eIF2a	Eukaryotic translation initiation factor 2A
ER	Endoplasmic Reticulums
ERAD	ER associated degradation
ERSE	ER Stress Response Element
FLT3	Fms-related tyrosine kinase 3
GADD34	Growth arrest and DNA damage-inducible protein
GCN2	General control nonderepressible 2

GRP78	Glucose-regulated protein 78
HOXB9	Homeobox B9
HIF1a	Hypoxia-inducible factor 1-alpha
HIV	Human Immunodeficiency Virus
HO-1	Heme oxygenase-1
HSPA5	Heat Shock Protein Family A (Hsp70) Member 5
HTLV-1	Human T-cell lymphotropic virus type 1
IAP	Inhibitor of Apoptosis Protein
IEL	intraepithelial lymphocytes
IH	Infantile Hemangioma
IFNγ	Interferon γ
ILC	Innate Lymphoid cells
IL	Interleukin
IRE1a	Inositol requiring enzyme-1 alpha
IRF	Interferon regulatory factor
IRS-1	Insulin receptor substrate 1
ISR	Integrated Stress Response
ITD	Internal tandem duplication
JNK	c-Jun N-terminal kinase
Lox1	lectin-type oxidized LDL receptor 1
LPS	Lipopolysaccharide
LTR	Long Terminal Repeat
MAPK	Mitogen-activated protein kinase
MCP1	Monocyte chemoattractant protein 1
M-CSF	Macrophage colony-stimulating factor
MDSC	Myeloid Derived Suppressor Cells
MEF	Mouse Embryonic Fibroblasts
MHV68	Gamma herpes virus 68
MITF	Microphthalmia-associated transcription factor

mTORC1	Mammalian target of rapamycin complex 1
M1/2	Activated/Alternately activated Macrophages
NFAT	Nuclear factor of activated T-cells
NF Kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural Killer cells
NKG2D	The Natural Killer Group 2D
NLRP1	NACHT, LRR, FIIND, CARD domain and PYD domains-containing protein 1
NRF2	Nuclear factor erythroid 2-related factor 2
PAMPs	Pathogen associated molecular patterns
PER1	Period Circadian Regulator 1
PERK	Protein kinase R (PKR)-like ER kinase
PpIr15a	Protein phosphatase 1 regulatory subunit 15A
PRMT1	Protein arginine N-methyltransferase 1
PHD	Prolyl-4-hydroxylase domain
OKC	Odontogenic keratocysts
RANKL	Receptor activator of nuclear factor kappa-B ligand
REDD1	regulated in development and DNA damage response 1
RIG	Retinoic acid-inducible gene
RTA	Replication and transcriptional activator
SIV	Simian Immunodeficiency Virus
SLC7A5	Solute carrier family 7 member 5
STAT3	Signal transducer and activator of transcription 3
Tbet	T-box expressed in T cells
TLR4	Toll-like receptor 4
TNFa	Tumor Necrosis Factor alpha
TRB3	Tribbles 3
TSC1/2	Tuberous sclerosis 1 /2
UBC	PolyUbiquitin-C precursor

ULBP1	UL16 Binding Protein 1

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Highlights

- ATF4 is activated as a result of cellular or oxidative stress and is a component of the unfolded protein response.
- Activation of ATF4 can affect immune cell growth and differentiation
- Viral and pathogen entry and evasion of immune detection can be regulated by ATF4.
- ATF4 can be targeted for therapeutic strategies against cancer, viral pathogenesis and inflammatory disorders

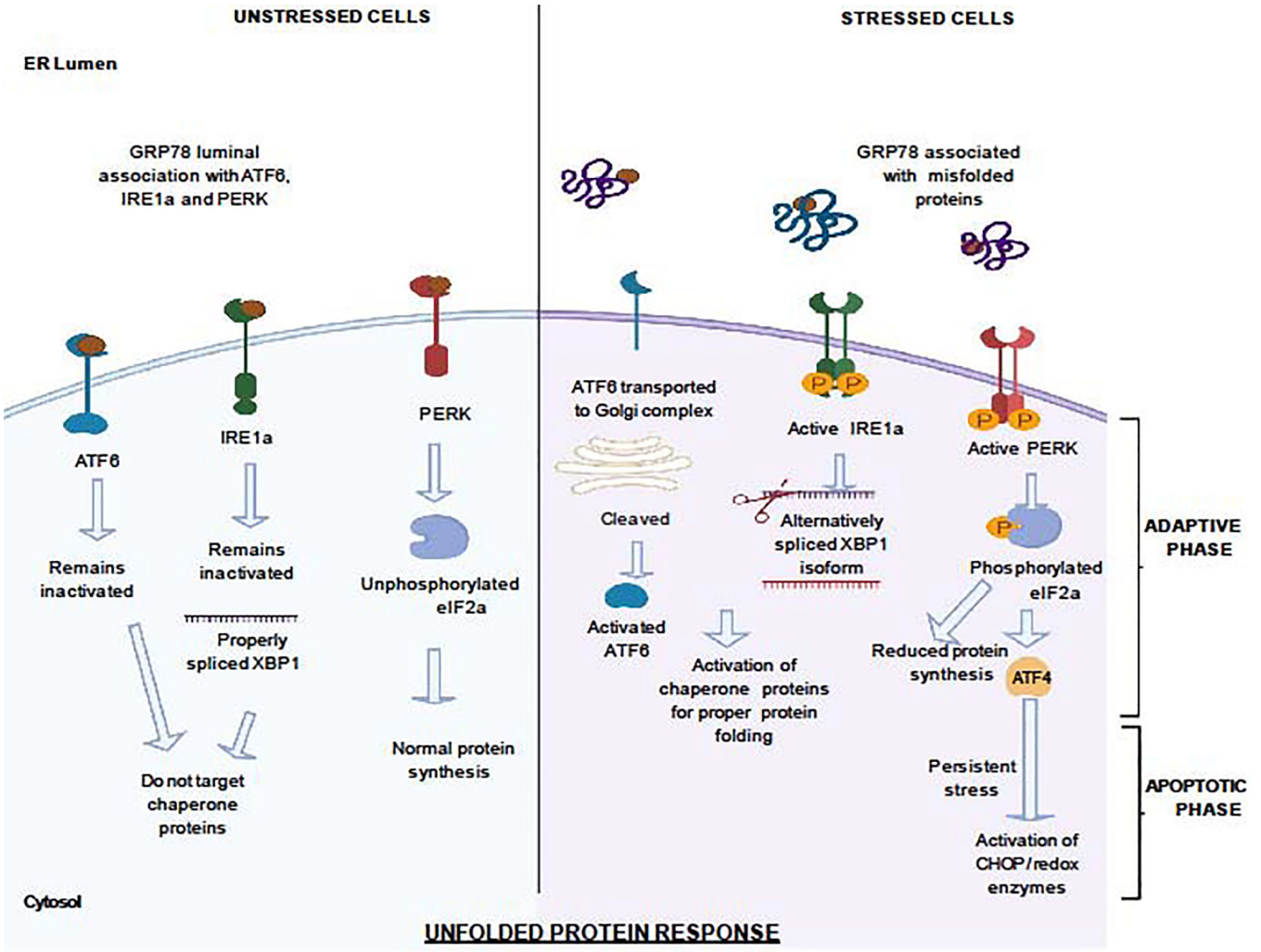

Figure1- Unfolded protein response (UPR) during cellular stress:

In unstressed cells, the different stress sensors like the protein kinase R (PKR)-like ER kinase (PERK), activating transcription factor-6 (ATF6), and inositol requiring enzyme-1 alpha (IRE1a) are inactive because they are luminally associated with 78 KDa glucose-regulated protein (GRP78) in the endoplasmic reticulum (ER). This allows unperturbed protein synthesis with no specialized expression of chaperone proteins. During stress conditions, GRP78 dissociates from PERK, ATF6 and IRE1a and preferentially binds to misfolded or unfolded proteins. Golgi localization signals of ATF6 become exposed translocating ATF6 to Golgi complex where it gets cleaved. The 50KDa cleaved ATF6 fragment can enter the nucleus and bind to ER stress response elements encoding chaperones and UPR modulators. IRE1a can be auto-phosphorylated to become active as a ribonuclease to alternatively splice X-Box DNA Binding Protein 1 (XBP-1) including a 26-nucleotide intron. The translational frameshift introduced results in a novel carboxy terminus that acts as a potent transcription factor for encoding chaperone proteins and restore ER homeostasis. Both ATF6 and XBP1-s can also upregulate GRP78 expression establishing a positive feedback loop to deal with the ER stress. PERK auto-phosphorylates to become active and can phosphorylate eIF2a. Phosphorylation of eIF2a reduces global protein

translation causing the cell cycle to arrest in the G1 phase. Simultaneously, selective translation of Activator of Transcription Factor-4 (ATF4) is allowed which upregulates expression of genes that can restore ER homeostasis. This is the adaptive phase of the UPR. ATF4 can transition from transcription of pro-survival genes to transcription of pro-apoptotic genes. On exposure to persistent stress, ATF4 can induce the expression of (CHOP). CHOP can mediate apoptosis through various processes like suppressing transcription of BCL2 family of anti-apoptotic proteins and activating pro-apoptotic proteins. Further, ATF4 can work with CHOP to dephosphorylate eIF2a while generating reactive oxygen species (ROS) by controlling gene expression of different redox enzymes. These processes lead to generation of ROS with an increased nascent protein load before restoring protein homeostasis maintaining the signal for apoptosis. This is the apoptotic phase of the UPR.

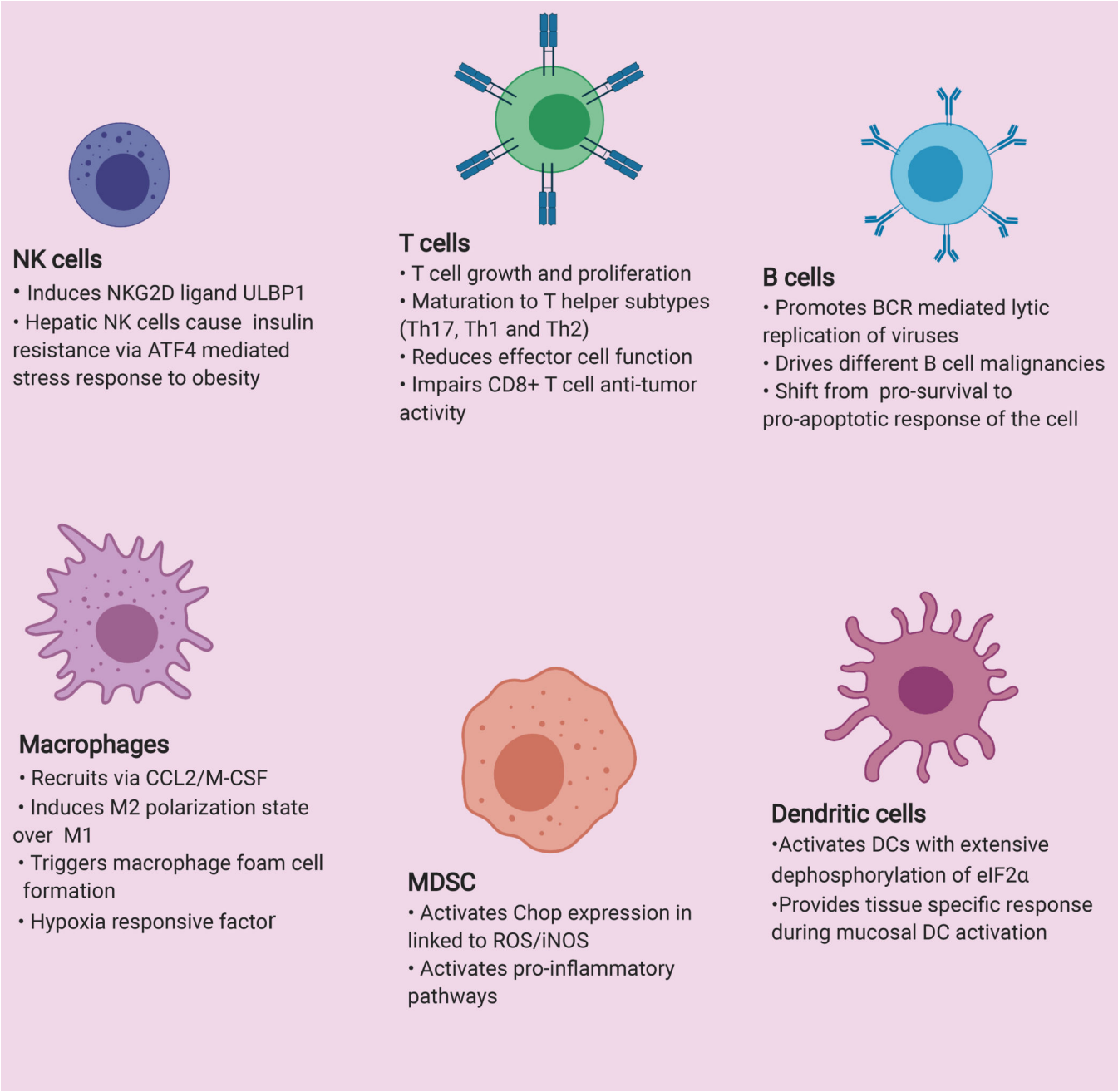

Figure 2- Overall regulation of immune cells by ATF4:

Interaction of ATF4 in regulating different immune cell types such as T cell, B cell, Natural Killer cell (NK cell), Macrophages, Dendritic cells and Myeloid Derived Suppressor Cell (MDSC).

Table 1-

Summary of Immune Cell Regulation by ATF4

Cell type	Phenotype	Major Finding	References
T cells	CFSE labelled CD4+ T Cells	ATF4 deficiency results in reduced Th1 response and increased Th17 response.	Ravindran et al.[44], Yang et al.[43]
	CFSE labelled mouse and human CD4+ T cells activated by DMSO expressing CD25, TH1 cells (IFN-γ + IL-4-), TH2 cells (IL-4+ IFN-γ-), or TH17 cells (IL-17+ IFNγ-)	HF selectively inhibits Th17 differentiation by activating Amino acid starvation response which targets ATF4.	Sundrud et al.[45]
	Human CD4+ T cells activated by antiCD3/CD28	HOXB9 targets ATF4 to suppress activated T cell function during amino acid insufficiency	Hayashi et al.[46]
	CD4+ T cells in Intestinal tissue and peripheral blood samples from rhesus macaques following SIV infection after early and chronic stages	Inhibition and enhancing ATF4 suppressed the induction of HIV expression and reactivated latent HIV, respectively.	Jiang G et al.[47]
	Jurkat Cells following HIV infection	Bystander stimulus of Tat on Jurkat cells resulted in time-dependent overexpression of major UPR markers	Campestrini et al.[48]
	Jurkat Cells following HTLV infection	All three members of the TORC family of transcriptional regulators but not ATF4 or other bZIP factors act as coactivators of Tax for LTR- driven expression.	Gachon F et al. [49], Siu et al.[50]
	SKmel28 cells and B16 melanoma mice model with the presence of a 219-geneTNFa response signature	ATF4 activates AXL and suppresses senescence to impose the MITF-low/AXL-high drug-resistant phenotype observed in human tumors.	Faletta et al.[35]
	CD8+ T cells sorted from tumor bearing mice.	Persistent ER stress activates ATF4 and results in Chop expression which can directly represses Tbet and therefore act as a major negative regulator of the effector function of tumor reactive $CD8^+$ T cells.	Cao et al.[37]
	Jurkat (clone E6-1) cells, Molt4-Bcl2 and Molt4-hyg cells, DEL, D011.10, Ht1080, and Ht1080mut cells	Specific induction of the PERK-elF2α-ATF3/4 cascade activating apoptosome in Molt4 T lymphoblastic T cells by Farnesol	Joo et al.[51]
	CD8+ T cells in C57BL/6 recipient mice receiving AMLMLL-PTD FLT3-ITD cells	Sorafenib-related IL-15 production caused metabolic reprogramming of leukemia-reactive T cells in humans via reduced expression of ATF4, thereby blocking negative regulation of interferon regulatory factor-7 (IRF-7) activation	Mathew et al.[52cg]
	Intraepithelial lymphocytes from inflamed Crohn's disease-like TNFΔARE/+ mice	Inflamed $TNF^{\Delta ARE/+}$ mice exhibited increased expression of ATF4 in addition to other ER stress proteins in a cytotoxic CD8αβ+ specific manner in the intraepithelial lymphocytes (IEL)	Chang et al. [53]
	PBMCs from patients diagnosed with ACLF, chronic hepatitis B (CHB-T) and chronic hepatitis B (CHB-A) differentiated into CD4+ T cells	ATF4 along with other ER stress proteins can be involved in epigenetic regulation of CD4+ T lymphocytes via the modification of H3K9ac in driving acute-on-chronic liver failure (ACLF)	Jin et al.[54]
Macrophages	Bone marrow-derived M2 like macrophages (BMDMs) from Lyz2-Cre+-G RP7 8f/f (Lyz- GRP78-/-) mice	GRP78 can regulate macrophage function and insulin resistance via ATF4 in diet induced obesity	Kim et al.[57]
	M2 macrophages	ATF4 promotes the expression of chemokine CCL2 that recruits macrophages contributing to endometrial tumor growth	Liu et al.[58]
	Raw264.7 macrophage cell line	ATF4 activity in breast cancer cells recruits macrophages via secretion of M-CSF	Liu et al.[59]
	Bone marrow-derived macrophages	RIG like receptor- based infection of West Nile Virus via the shift to M1 phenotype via suppression of M2 phenotype by down regulating ATF4	Stone et al.[60]

Cell type	Phenotype	Major Finding	References
	Human monocytes from healthy blood donors, differentiated into MDM.	ATF4 was found to be the hypoxia responsive factor in macrophages early after exposure	Elbarghati et al.[61]
	M2-polarized macrophages (CD68+/CD163+)	Positive regulation of hypoxia- stimulated ATF4 activation leads to M-CSF based recruitment of M2 polarized macrophages	Xia et al.[62]
	M2-polarized macrophages (CD68+/CD163+)	Hypoxia elevates ATF4 expression that stimulates RANKL and contributes to OKC development	Zhong et al.[63]
	PBMCs from healthy donor blood were differentiated into bone marrow-derived macrophages	Cathepsin inhibition can result in mitochondrial stress and ROS production which triggers the ATF4-CHOP pathway to conduct autophagy and form macrophage foam cells in artherosclerosis	Weiss-Sadan et al.[64]
	Mouse macrophage cell line Raw 264.7 and peritoneal and lung macrophages fromOo ATF4+/+ wild-type (WT) and ATF4+/- heterozygous mice in the C57BL/6j background	ATF4 plays an underlying role in pathogenesis of macrophage dysregulation and immune-toxicity of arsenic via down-regulation of CD11 b expression associated with the reduced phagocytic functions of peritoneal and lung macrophages.	Srivastava et al.[93]
	RAW264.7 macrophage cell line	siRNA mediated knockdown of CHOP attenuated atherogenic lipid-mediated induction of proteins and genes implicated in macrophage mediated inflammation, ER stress (ATF4 and ATF6), and apoptosis (CHOP).	Zahid et al.[65]
B cells	IgG for MHV68 lytic antigen in MHV68-transformed SL-1 cells	ATF4 inhibits promoter activity of the MHV68 lytic switch transactivator RTA promoting BCR- mediated lytic replication of gamma herpes virus 68 (MHV68)	Zhou et al.[67]
	B- cell lymphoma cell lines and cells isolated from mice	Artesunate treatment induces anti-tumor activity to treat B cell lymphoma by suppressing ATF4	Vatsveen et al.[68]
	SV40-immortalized WT and Atf4-/- MEFs in C57BL/6J *Btg1-/-* and *Btg2-/-* mice	Methylation of ATF4 by PRMT1 to allow transcription of a subset of ATF4 target genes leading to increased apoptosis in contrast to survival is regulated by BTG1	Yuniati et al.[69]
NK Cells	NK cells from normal donors and Type II diabetes patients	ER stress contributes to downregulation of NKG2D in-vivo altering NK cell function in Type II diabetic patients.	Berrou et al.[76]
	shXbp1 MODE-K cells with splenic NK cells	Innate immune sensing of ER stress via Chop is important for understanding the mechanism of intestinal inflammation.	Hosomi et al.[77]
	NK cells from Human liver cell lines (HL-7702) treated with OPN, C57BL/6 male mice under HFD	Obesity can induce Hepatic NK cells to produce ATF4 mediated ER stress response resulting in insulin resistance	Wu et al.[78]
Dendritic Cells	Mouse bmDCs activated with pl:C	DCs mount a specific integrated stress response during which ATF4 and GADD34 is expressed with an extensive dephosphorylation of the translation initiation factor elF2α during DC activation.	Clavarino et al.[80]
	Lung, intestinal and mucosal cDCs	ER stress response pathways can mediate tissue specific activation of different mucosal DCs via ATF4.	Tavernier et al.[81]
	DC2.4 cells, CD11c+ dendritic cells from spleens of sepsis induced C57BL/6J mice	Sestrin2 inhibits PERK-ATF4-CHOP mediated cell death pathway upon high mobility group box-1 protein stimulation rendering a protective effect on DCs.	Wang et al.[82]
MDSCs	Splenic and tumor-MDSCs recovered from tumor bearing mice using anti-Gr1 Ab	Chop expression in MDSCs was shown to be driven by ATF4 in the presence of tumor linked reactive oxygen and nitrogen species.	Thevenot et al.[87]
	CD8+ T cells from B6.Gcn2fl/ flxLyz2+/Cre mice	GCN2 can promote translation of ATF4 and increase activation of pro-inflammatory MDSCs, macrophages and IFNy expression in intra-tumoral CD8+T cells adversely affecting the tumor microenvironment.	Halaby et al.[88]
General/ Innate immunity	*A. fumigatus* strain 3.0772, C57BL/6 female mice, Human corneal epithelial cells (HCECs), THP-1 macrophages;	ATF4 expression was shown to be dependent on TLR4, LOX-1 expression, and MAPKs pathway and therefore involved in host antifungal immune response	Zhang et al.[92]

Cell type	Phenotype	Major Finding	References
	Immortalized mouse retinal endothelial cells, and C57BL/6 J mice	ATF4 is critical in regulation of Monocyte chemoattractant protein 1 (MCP1) in retinal and brain microvascular endothelial cells contributing in inflammation- related endothelial injury in diseases such as diabetic retinopathy.	Huang et al.[94]
	HEK-293FT, RAW264.7, Murine primary macrophages were thioglycolate-elicited and removed from wild-type (WT) or TLR4-knockout (KO) C57BL/6 mice	Knocking down ATF4 in RAW 264.7 macrophage cells decreased NRF2 expression and its nuclear translocation, reduced HO-1 expression and increased nitric oxide production suggesting that ATF4 pathway is involved in parasite survival and progression against Leishmania amazonensis infection.	Dias-Teixeira et al.[95]
	THP-1, K562 and Jurkat cells	During ER stress, ATF4 directly binds to NLRP1 promoter (core inflammasome component) and reaulate inflammation.	D'Osualdo et al.[96]