Graphical Abstract

Review Article

Tripping on TRIB3 at the junction of health, metabolic dysfunction and cancer

*Debasis Mondal, Aditi Mathur, Partha K. Chandra

Department of Pharmacology, Tulane University School of Medicine

1430 Tulane Avenue, New Orleans, LA 70112. USA

Keywords: TRIB3, metabolic syndrome, cancer, ER-stress, PI3K/AKT/mTOR, autophagy

*** Address correspondence to:**

Debasis Mondal, Ph.D.

Dept. of Pharmacology

Tulane University School of Medicine

1430 Tulane Avenue

New Orleans, LA 70112, USA

Email: dmondal@tulane.edu

Abstract

Metabolic diseases like obesity, atherosclerosis and diabetes are frequently associated with increased risk of aggressive cancers. Although metabolic dysfunctions in normal cells are manifested due to defective signaling networks that control cellular homeostasis, malignant cells utilize these signaling networks for their increased survival, growth and metastasis. Despite decades of research, a common mechanistic link between these chronic pathologies is still not well delineated. Evidences show that the unfolded protein response (UPR) and the endoplasmic reticulum stress (ERS) pathways are often dysregulated in both metabolic diseases and cancer. The UPR also triggers coordinated signaling with both PI3K/AKT/mTOR and Autophagy pathways in order to promote stress-adaptive mechanisms. Whereas, uncontrolled UPR and the resultant ERS escalates cells towards metabolic dysfunctions and ultimately cell death. In this review, we will discuss findings that implicate a crucial role for the multifunctional ERS-induced protein, TRIB3. The 'pseudokinase' function of TRIB3 facilitates the inactivation of multiple transcription factors and signaling proteins. The MEK1 binding domain of TRIB3 enables it to deactivate multiple MAP-kinases. In addition, the COP1 motif of TRIB3 assists ubiquitination and proteasomal degradation of numerous TRIB3 associated proteins. The most well studied action of TRIB3 has been on the PI3K/AKT/mTOR pathway, where TRIB3-mediated inhibition of AKT phosphorylation decreases insulin signaling and cell survival. Conversely, cancer cells can either upregulate the AKT survival pathway by suppressing TRIB3 expression or alter TRIB3 localization to degrade differentiation inducing nuclear transcription factors such as C/EBP α and PPAR γ . The gain-of-function Q84R polymorphism in TRIB3 is associated with increased risk of diabetes and atherosclerosis. TRIB3 acts as a crucial 'stress adjusting switch' that links homeostasis, metabolic disease and cancer; and is being actively investigated as a disease biomarker and therapeutic target.

Table of Contents

I. A long sought-after connection between metabolic dysfunction and cancer

- 1.1. Metabolic diseases increase cancer-associated morbidity and mortality
- 1.2. Inflammation: a common etiology in chronic diseases

II. The multifunctional Tribbles

- 2.1. Discovery of mammalian TRIB proteins
- 2.2. Differential effects of TRIBs on second messenger signaling

III. The mammalian TRIB3 protein

- 3.1. Multimodal actions of TRIB3
- 3.2. Functional motifs of TRIB3

IV. TRIB3, a stress-induced factor

- 4.1. Metabolic stress: a balance between homeostasis and disease
- 4.2. Three UPR cascades control the progression of ERS

V. TRIB3 regulates multiple stress response pathways

- 5.1. Cross-talks between PI3K/AKT/mTOR, autophagy and TRIB3
- 5.2. Cross-talks between NF- κ B, MAPK and TRIB3

VI. TRIB3 association with metabolic dysfunctions

- 6.1. TRIB3 suppresses insulin signaling and glycogen storage
 - 6.1.1. *TRIB3 overexpression in insulin resistance and diabetes*
 - 6.1.2. *TRIB3 is associated with atherosclerotic plaque rupture*
 - 6.1.3. *TRIB3 is associated with β -cell dysfunction and death*
 - 6.1.4. *TRIB3 Q84R polymorphism in diabetes and atherosclerosis*
- 6.2. TRIB3 is associated with numerous diabetes-associated diseases
 - 6.2.1. *Hyper-homocysteinemia*
 - 6.2.2. *Non-alcoholic fatty liver disease*
 - 6.2.3. *Diabetic nephropathy*

VII. Linking TRIB3 expression in obesity and cancer

- 7.1. TRIB3 expression in adipocyte differentiation and visceral obesity
- 7.2. Potential of targeting TRIB3 in aggressive cancers

VIII. Perspectives

IX. References

X. Figure Legends

I. A long sought-after connection between metabolic dysfunction and cancer.

1.1. Metabolic diseases increase cancer-associated morbidity and mortality. Chronic metabolic diseases like obesity, type-2 diabetes, atherosclerosis, and cardiovascular disease (CVD) are becoming increasingly prevalent worldwide [1-4]. Both hyperinsulinemia and hyperglycemia contribute to the progression of obesity and diabetes, and insulin resistance, manifested due to decreased insulin receptor signaling, is the primary risk factor for these metabolic disorders [5, 6]. Interestingly, metabolic diseases are also frequently associated with poorer cancer outcomes [7, 8]. In the past few decades, a number of studies have documented a clear link between the metabolic syndromes and higher morbidity and mortality due to different malignancies [9-11]. However, the crucial mechanism(s) involved in linking these chronic pathologic manifestations is not properly understood. As early as 1995, Steenland *et al*, showed that men with diabetes present with a 39% higher risk of developing colorectal and prostate cancer [12]. Calle *et al*. (2003) published finding from the multicenter Cancer Prevention Study (CPS) which followed more than one million adults during 1982 to 1996, and clearly demonstrated that obese men and women had a 40-80% increased threat of dying from cancers [13, 14]. The danger of having aggressive pancreatic, breast and colorectal cancers are reported to be amplified in patients with high body mass index (BMI) and several meta-analyses in patients with diabetes also showed significantly higher cancer mortality, as compared with nondiabetic individuals [15, 16]. Indeed, the clinically approved glyburide, metformin has provided better clinical outcome in diabetic patients with advanced cancers [17]. Hence, a thorough understanding of the long sought-after relationship between metabolic diseases and cancers will not only provide early biomarkers for disease progression, but will also elucidate novel therapeutic targets to decrease cancer-associated complications. Furthermore, since tremendous increases in metabolic syndrome are being reported in younger adults [4], which makes them

more susceptible to malignancies later in life, studies on the common etiologies in metabolic diseases and cancer are garnering a lot of attention [7, 17, 18-22].

Deleterious consequences of chronic inflammation and oxidative stress are known to increase tumor progression and metastasis, and also facilitate tumor resistance to both chemotherapy and radiotherapy [7, 20, 23-28]. Studies have documented that the visceral adipose tissue secreted inflammatory cytokines can promote insulin resistance in vascular cells [29-32]. Metabolic complications of insulin resistance make individuals more susceptible to chronic oxidative stress, neoplastic transformation and aggressive tumor growth. A number of studies have also demonstrated that second messenger signaling via the insulin and insulin like growth factor (IGF) receptors play a crucial role in numerous other chronic diseases such as autoimmunity, arthritis, alzheimer's disease and aging [33-36]. Thus, it is becoming apparent that the chronic effects of inflammation in disrupting stress-adaptive pathways in both normal and malignant cells may influence progression of these chronic diseases.

1.2. Inflammation: a common etiology in chronic diseases. Obesity induced adipokines, inflammatory cytokines, leptin, proteolytic enzymes, and endogenous sex steroids, are known to suppress the anti-inflammatory actions of insulin. The resultant activation of vascular endothelium and decreased vasodilation of smooth muscle cells increases blood pressure and causes hypertension [32, 37, 38]. Increased adhesion of leukocytes and platelets and the ensuing atherosclerotic thrombus formation further fosters inflammatory stress and insulin resistance of the vasculature. Adipose tissue infiltrated macrophages and foam cells can also produce a state of chronic oxidative stress and inflammation, which further promote the chronic metabolic dysfunctions [39, 40]. Interestingly, the deleterious effects of insulin resistance, inflammation and oxidative stress have also been implicated in both oncogenic transformation of normal cell [41, 42] and in increased proliferation and metastasis of tumor cells, as shown by us [43] and others [20, 28, 44, 45]. Furthermore, since insulin resistance increases both

estrogen and testosterone levels by decreasing SHBG (sex hormone binding globulin) metabolic diseases can also augment the growth of endocrine tumors like breast and prostate cancers (7, 23, 43]. Therefore, insulin resistance is postulated to be a common link and comorbidity in both metabolic diseases and cancer. Indeed, insulin-induced glucose uptake is dysregulated in chronic hyperglycemia and hyperinsulinemia [46, 47].

The mitochondrial NAD-dependent deacetylase sirtuin-3 (SIRT3) can help maintain the protective effects of insulin in both skeletal muscles and adipose tissue [48]. Interestingly, a direct role of the AKT pseudokinase, TRIB3 in regulating insulin sensitivity and nutrient metabolism has been documented [49]. It is well established that normal cells rely on mitochondrial oxidative phosphorylation of glucose to generate adenosine 5'-triphosphate (ATP). However, cancer cells utilize an alternate phenomenon known as the “Warburg effect”, where aerobic glycolysis and lactate production is used as the primary source of ATP [50, 51]. As early as in 2006, Schwarzer *et al.* had shown that TRIB3 can regulate glucose metabolism and glycolysis, and these studies showed that TRIB3 functions as an indicator of nutrient starvation via targeting the PI3K/AKT pathway [52]. Numerous recent studies have also shown a direct role of both hypoxia [53] and the PI3K/AKT pathway [54] in tumor growth and therapeutic resistance. As will be discussed later, TRIB3 is now well-accepted inhibitor of AKT [55, 56] and TRIB3 gene expression is induced following hypoxic stress [57, 58].

Numerous control mechanisms and stress-adaptive pathways exist in cells to prevent the aberrant intracellular signaling during insulin resistance, which help maintain homeostasis in both normal and malignant cells [59-62]. These master regulatory pathways judiciously balance the nutrient-sensing and stress-inductive machinery that dictate homeostasis, cellular dysfunctions, or survival & growth. Accurate identification of this ‘molecular regulatory switch’ will be of significant importance as both an effective disease biomarker and a potent therapeutic target. In the following sections, we will present a

number of independent studies indicating that the mammalian ‘Tribbles’ proteins are at the nexus of these metabolic pathways and may function as the ‘molecular regulatory switch’. We will focus on the most multifunctional of these Tribbles proteins, i.e. TRIB3, and present findings that demonstrate its role in regulating disease progression in both metabolic syndrome and cancer [63-68].

II. The multifunctional Tribbles

2.1. Discovery of mammalian TRIB proteins. Tribbles protein, originally identified in *Drosophila melanogaster* (fruit fly), was shown to be an evolutionarily conserved protein that regulates multiple cellular processes [69, 70]. Initial studies showed that loss of Tribbles can increase proliferation of fruit fly embryos and its overexpression decreased cell cycle progression & enhanced morphogenesis [71]. Molecular mechanistic studies revealed that Tribbles overexpression arrested cells in the G2 phase of cell cycle by enhancing proteasomal degradation of two *Drosophila* cell cycle regulating phosphatases, String and Twine [72]. These proteins were later found to be homologs of mammalian cyclin dependent kinase, Cdc25 [73]. Since Cdc25 activates the major mitotic kinase Cdk1, its degradation decreases mitosis and growth of mammalian cells. Tribbles was also found to increase the ubiquitination and proteasome-mediated degradation of the *Drosophila* protein, Slbo (slow border cells) [74], later identified as a homolog of the mammalian transcription factor C/EBP (CAAT enhancer binding protein) [75]. Indeed, multiple C/EBP transcription factors, e.g. alpha, beta, delta (α , β and δ) play crucial roles in lineage specific differentiation of endothelial, smooth muscle and adipose cells [76-78]. These C/EBP proteins are also well-known regulators of cancer cell growth [79, 80]. Indeed, Bowers *et al.* (2002) was the first to show that the mammalian tribbles homolog, TRIB3 (a.k.a. SKIP3) is overexpressed in human tumors and is directly associated with cellular dysfunctions [81].

Mechanistic understanding of the numerous functions of *Drosophila* Tribbles fueled intense research in this field, which facilitated the discovery of three mammalian homologs, TRIB1, TRIB2 and

TRIB3 [82, 83]. These early studies on TRIB proteins were found to decrease cell migration and increase differentiation of different mammalian cells. Subsequent studies in multiple laboratories documented a direct role of mammalian TRIBs in cell proliferation, metabolism, and oncogenic transformation [84-89]. Interestingly, further studies on the TRIB homologs also divulged significant differences in their amino acid contents and protein tertiary structures. Furthermore, although the *TRIB* genes were found to code for similar functional domains, distinct variations in their functional activities were clearly evident in different laboratories. Homology between TRIB1 and TRIB2 was found to be as high as 71.3 %. However, TRIB3 only showed 53.3% and 53.7% homology with TRIB1 and TRIB2, respectively [83]. The expression of TRIB proteins and their subcellular localization also varied in different tissues, as well as in different disease models [49, 56, 64, 65, 84, 86, 87]. TRIB1 was observed to preferentially localize to the nucleus and TRIB2 was usually detected in the cytoplasm; however, TRIB3 expression was documented in both cellular compartments. As compared to TRIB1 and TRIB2, both subcellular localization functional association studies clearly implicated a more global importance of TRIB3 in different diseases. This has been presented in two highly cited review articles by Prudente *et al.* (2009 & 2012) [90, 91]. Findings within the last decade emphasized that the multimodal actions of TRIB3 coordinate important metabolic processes including glucose and lipid metabolism, inflammation, survival, oxidative stress, apoptosis, and most importantly, tumorigenesis. The crucial importance of TRIB3 is further underscored from the recent identification of a small molecule ABTL0812 that upregulates TRIB3 and its entry into several antitumor clinical trials [56].

2.2. Differential effects of TRIBs on second messenger signaling. Independent studies on different TRIB isoforms have provided a general consensus that TRIB protein expression is critically regulated by cellular stress due to either overstimulation or deprivation of nutrients, like glucose, amino acids and free fatty acids [83, 84, 93-98]. Both transcriptional regulation of *TRIB* genes and post-translational

modification of TRIB proteins have been documented. In addition, functional association of TRIBs with other cellular proteins and their differential subcellular localization in cytosol and nucleus ultimately dictates their actions. Activation of signaling via inflammatory cytokines such as TNF α (tumor necrosis factor-alpha), IL-3 (interleukin-3) and HIF-1 α (hypoxia inducible factor-1-alpha) as well as depletion of growth factor signaling from NGF (nerve growth factor) and IGF-1 (insulin like growth factor-1) can upregulate the expression of different TRIB isoforms. Indeed, immune-histochemical (IHC) analysis of tissues from patients with metabolic diseases revealed increased expression of multiple TRIB proteins, and most interestingly, both changes in expression (up or down regulation) and subcellular localization (cytosol vs. nucleus) was frequently seen [66, 67, 99]. TRIB1 overexpression has often been associated with metabolic dysfunctions in vascular tissues [100]. In endothelial cells, TRIB1 plays a direct role in regulating both PKB (protein kinase B/AKT) and RAR (retinoic acid receptor) signaling [101]. In smooth muscle cells, overexpression of TRIB1 inhibits MAPK (mitogen activated protein kinase) mediated activation of transcription factor AP-1 (activated protein-1) [85]. Furthermore, an important role of TRIB1 in differentiation of M2-like macrophages was recently shown by Satoh *et al.* (2013) [102]. Interestingly, although TRIB2 levels were not significantly augmented in vascular endothelium obtained from obese or diabetic patients, its induction has been associated with both CNS (central nervous system) dysfunctions and tumorigenesis. Increased circulating levels of antibodies against TRIB2 were found in individuals with narcolepsy [103]. TRIB2 expression was also shown to be augmented in lymphocytes from AML (acute myelogenous leukemia) patients [104]. Indeed, TRIB2 has been directly linked to oncogenic transformation in AML [104], liver cancer [105] and melanoma [106]. Similar to TRIB2, TRIB3 levels were significantly associated with tumor nodes, as compared to the surrounding normal stroma. However, unlike TRIB2, the expression of TRIB3 was found to be either upregulated or downregulated in both primary tumors and in different cancer cell lines [68, 89, 96-98, 107-111].

Remarkably, although TRIB3 message is ubiquitously expressed in both mesenchymal and hematopoietic cells, TRIB3 protein levels were more precisely regulated in both a context- and microenvironment-dependent manner. Brisard *et al.* (2014) provided a very intriguing observation in primary oocytes, where differential expression and subcellular localization of all three TRIB proteins was observed during the different pre-ovulatory periods in cumulus cells [112]. These findings clearly suggested that the mammalian TRIBs act as a central node involved in fine tuning of multiple cellular processes, in both normal and transformed cells. Since TRIB3 is the most well studied member of this family, in the following sections we will discuss the multimodal actions of TRIB3 and its role in causing different pathophysiologic manifestations. We will also provide an overview on how TRIB3 expression is exquisitely regulated during homeostasis and stress. Lastly, we will also discuss the findings that show TRIB3 as a disease biomarker and a novel therapeutic target in metabolic diseases and cancer.

III. The mammalian TRIB3 protein

3.1. Multimodal actions of TRIB3. Studies have recognized the crucial functions of TRIB3 in cells of both mesenchymal [65, 91, 113-116] and hematopoietic lineages [94, 117-119]. Although the deleterious actions of TRIB3 in metabolic tissues are focused on its potent ability to dysregulate insulin signaling [65, 91, 120, 121] multiple other functions of TRIB3 are also evident in diverse cell types, such as apoptosis in both neuronal cells [52] and pancreatic β -cells [88] and glucose production in hepatocytes [122, 123]. Studies have also shown that TRIB3 can promote ubiquitination and degradation of different cell cycle regulatory proteins [124] and this attribute of TRIB3 also dictates its own degradation via the association with an E3-ubiquitin ligase, SIAH1 [125]. In contrast, TRIB3 is found to protect HEK293 cells against the growth inhibitory and cytotoxic effects of the ER-stress (ERS)-induced transcription factor, ATF4 [126]. Interestingly, although TRIB3 increased neuronal cell death due to nutrient deprivation [127] it was associated with increased survival in mast cells [117]. These dissimilar functions of TRIB3 have been

linked to a cross-talk between TRIB3, AKT and the FoxO regulated signaling axis [128]. Due to its crucial role in cell cycle, proliferation & differentiation, recent studies also suggest that TRIB3 may be important in maintaining the pluripotency of normal stem cells [112, 129, 130] and in the EMT (epithelial-to-mesenchymal transition) phenotype of cancer stem cells (CSCs) [131-133]. Interestingly, depending on the stage of tumor progression TRIB3 expression varied in different solid tumors, e.g. lung, colon, esophageal, and breast cancers [68, 96, 134, 135]. These crucial findings on TRIB3 mediated regulation of cell cycle progression and mitogenesis in both normal and transformed cells have thus sparked new directions in research to understand its function in different disease manifestations, as well as its utility as a biomarker of indolent *vs.* aggressive disease. The ability of TRIB3 to sensitize lymphoma cells to sorafenib-induced apoptosis [136], in increasing the action of autophagy-mediated cell death in glioma cells [108, 137] and in augmenting ERS mediated chemosensitization of prostate cancer cells to taxols [110] has fostered substantial therapeutic implications for this enigmatic protein, as well [56].

3.2. Functional motifs of TRIB3. The TRIB3 mRNA is coded from six exons located in human chromosome-20 (20p13-p12.2) which generates a 358 amino acid protein of approximately 65 KDa [138-140]. Protein sequence analysis showed that TRIB3 has three functional motifs; i.e. a central kinase-like domain, and both N-terminal and C-terminal protein-binding domains with distinct functionalities **[Figure-1]**. Earlier studies had focused on the central serine/threonine kinase-like domain of TRIB3 [138]. Although this region of TRIB3 contains the kinase catalytic core, it is divergent at the consensus ATP-binding pocket and thus does not possess any kinase activity [64, 95]. TRIB3 was thus classified as a ‘pseudokinase’ similar to ILKs (integrin-linked kinases) and JAKs (janus tyrosine kinases [141, 142]. Studies have shown that TRIB3 can bind to a number of kinase-dependent proteins and dysregulate their function by negatively regulating their phosphorylation, thus alter multiple signal transduction pathways [64, 95, 138-140]. TRIB3 drastically affected cell-fate determination by negatively affecting the

activation of both PI3K/AKT [67, 95] and Notch [107, 135], which are crucial survival pathways in cells undergoing stress. Furthermore, by suppressing the functional activation of lineage specific transcription factors like C/EBP (CAAT enhancer binding protein) and PPAR (peroxisome proliferating activated receptor) TRIB3 was also shown to alter differentiation of endothelial cells, myocytes and adipocytes [77, 87, 94, 143, 144].

In addition to the well-studied ‘pseudokinase’ domain, both the N-terminal and C-terminal domains of TRIB3 have also been associated with functions that act in additive or synergistic manner to further fine tune numerous signal transduction cascades. The N-terminal domain is high in serine and proline content, a characteristic of the PEST sequence [i.e. proline (P), glutamic acid (E), serine (S), and threonine (T)] which is involved in the degradation of TRIB3 via both SIAH1 [125] and cdh1 [144]. Interestingly, combined action of this PEST sequence and the ubiquitin ligase function of TRIB3 can also facilitate proteasomal degradation of multiple client proteins and crucial cellular transcription factors such as ATF4, C/EBP, PPAR and I κ B α [57, 126, 143, 145-147]. A nuclear localization signal has also been detected at this N-terminal region of TRIB3 [96] and thus TRIB3 may be able to alter transcription factor activity in both the cytosol and nucleus. Interestingly, Hua *et al.* (2015) recently reported that TRIB3 is a stress-induced protein that mediates reciprocal antagonisms between autophagic and proteasomal degradation systems, which facilitates the connection of insulin (or IGF-1) signaling to tumor promotion via the induction of autophagy and lysosomal degradation [148]. This important finding also documented that TRIB3 interactions with SQSTM1 can decrease the degradation of this autophagic receptor, and increased the accumulation of other ubiquitinated proteins. Furthermore, siRNA mediated decrease in TRIB3 was able to restore autophagy, and most interestingly, TRIB3 knockdown was successful in attenuating tumor growth and metastasis [148]. In contrast to several previous studies in tumor specimen, where lower TRIB3 and higher phosphorylated-AKT levels were associated with poor patient outcome

[66, 89, 134] the above finding by Hua *et al.* (2015), showed that both TRIB3 expression and insulin signaling were activated in cancer patients with a negative prognosis [148]. These investigators also suggested that the blocking of TRIB3-SQSTM1 interactions by using small molecule inhibitors may be a novel strategy against cancers, especially in patients with diabetes [148].

The C-terminal domain of TRIB3 contains two conserved sequences, i.e. a binding site for mitogen activated protein kinases (MAPKK) termed the MEK1 domain [95, 107, 140] and the COP1 domain (constitutive photomorphogenic protein 1) which regulates ubiquitin ligation of TRIB3 associated proteins [80, 105, 125, 149]. The MEK1 site mediates interactions with multiple MAPKKs, and a high throughput kinase inhibitor screen revealed that TRIB3 is a potent inhibitor of MAPK-ERK/TGF β pathway in breast cancer cells [107]. By facilitating the ubiquitin binding at the COP1 site, TRIB3 can regulate proteasomal degradation and half-life of multiple client proteins. Indeed, TRIB3 was able to suppress insulin-induced adipocyte differentiation by negatively regulating PPAR γ transcriptional activity [143]. An *in vivo* study in a rat model of insulin resistance also showed that knockdown of TRIB3 can improve insulin sensitivity through PPAR γ activation [145]. In tumor models of AML [146] and liver cancer [147] TRIB3 promoted degradation of both C/EBP α and NF- κ B to exert its anti-tumor effects. Interestingly, Aynaud *et al.* (2012) also documented that TRIB3 can interact with the DNA mutator cytidine deaminase APOBEC-3A (A3A) resulting in its proteasome-independent degradation [148]. Co-transfection of A3A and TRIB3 expression vectors reduced nuclear DNA editing and suggested that TRIB3 may be a guardian of genomic integrity, which is disrupted during the process of oncogenic transformation. Therefore, the last few years have further illuminated our understanding of how TRIB3 functions at the juncture of homeostasis, metabolic disease and cancer.

IV. TRIB3, a stress-induced factor

4.1. Metabolic stress: a balance between homeostasis and disease. Microenvironmental stressors, which are activated following either nutrient excess or nutrient deprivation, can alter cellular homeostasis [150-153]. Increased protein synthesis is needed to cope with increased metabolic demand during stress in both normal and transformed cells, and thus, a coordinated protein folding by the endoplasmic reticulum (ER) and increased protein degradation by the proteasome and lysosome are of significant importance towards maintaining homeostasis [59, 119, 137, 152, 153]. The proteolytic systems recognize and destroy misfolded or damaged proteins and are essential in basic cellular processes including cell cycle modulation and second messenger signaling. Although the equilibrium is preserved under physiologic conditions, it is severely affected when unfolded and misfolded proteins accumulate. Therefore, an adaptive signaling pathway called the unfolded protein response (UPR) is initiated to re-establish protein balance in cells undergoing stress. However, compromised ER function can also initiate signaling networks that suppress the stress-adaptive mechanisms, which results in cellular dysfunctions that promote uncontrolled UPR and progression towards ER-stress (ERS). Prolonged or severe ERS, and an inability of cells to sustain the UPR, then subverts the survival pathways and initiates programmed cell death. As will be discussed in the later sections, TRIB3 has a crucial function in regulating whether cells sustain UPR and promote homeostasis or then progress towards ERS and promote cell death. Comprehensive reviews on the role of ERS pathways in metabolic diseases and cancer had been provided before [154, 155], and hence, it is only discussed in brief in the following sections.

Normal cells have a low threshold for stress-adaptation and show low levels of UPR. Chronic UPR activation results in severe metabolic dysfunctions in normal cells [59, 152]. Therefore, strategies to decrease the constitutive UPR, and its progression towards ERS, are being investigated as therapy against the metabolic syndrome [48, 60, 66, 148]. On the other hand, due to their increased protein synthesis, aggressive cancer cells have constitutive UPR. Both UPR and autophagy pathways are well-known to

promote cancer growth, metastatic progression and therapeutic resistance [156-158]. However, the exploitation of this overactive UPR to promote ERS-induced apoptosis in cancer cells is also becoming a promising approach in multiple tumor models [110, 132, 133, 159]. Since TRIB3 is upregulated by several stress-induced transcription factors [81, 95, 160] and since it is notorious in regulating the balance between UPR and ERS [161], the targeting of TRIB3 may also be a novel approach [58, 110]. Below, we are presenting a short description of how TRIB3 expression is fine-tuned by cross-talks with other intracellular signaling pathways, a proper understanding of which will provide better treatment strategies.

4.2. Three UPR cascades control the progression of ERS. Defective proteins are subjected to "proof-reading" and are rapidly degraded by proteases, and disruption of this protein homeostasis can result in chronic diseases. The stress-adaptive phase of UPR assists in increased protein folding by numerous chaperone proteins, enhanced protein degradation by proteasomal machinery, and decreased protein translation in the rough ER [154, 155] **[Figure-2]**. Three downstream UPR transducer cascades are initiated following proteotoxic stress, these are: (i) the ATF6 (activating transcription factor-6) pathway, (ii) the IRE1 (inositol requiring enzyme-1) pathway, and (iii) the PERK (protein kinase RNA-like endoplasmic reticulum kinase) pathway. One of the most crucial molecular chaperones responsible for proper folding of proteins is Grp78 (Glucose regulated protein 78; a.k.a. BiP) [162]. Under homeostatic conditions, the ER membrane-associated Grp78 is bound to all three of the above UPR transducers (ATF6, IRE1 and PERK). However, following metabolic stress, Grp78/BiP dissociates from these transducers and initiates the downstream cascades that either facilitate stress-adaptation or promote stress-induction [154, 155]. Dissociation of ATF6 (arm-1) from Grp78 causes its translocation into the golgi apparatus where it is cleaved to its active form by two site specific proteases (SP-1 and SP-2). Active ATF6 then translocate into the nucleus and enhances transcription of multiple chaperones like Grp78 and Grp94. Therefore, the ATF6 axis primarily functions in a pro-survival capacity to counteract

metabolic stress [163]. In the second UPR cascade, dissociation from Grp78 activates the endoribonuclease action of IRE1, which causes the splicing of XBP-1 mRNA (X-box binding protein-1). The spliced XBP1 mRNA codes for a transcription factor that induces genes needed for increased protein folding capacity, and thus again help promote cell survival. In mice, hepatic fatty acid and triglyceride metabolism was shown to occur through XBP1 [164]. Importantly, Duan *et al.* (2015) showed that several miRNAs regulate XBP1 expression and progression of cardiac hypertrophy and heart failure *in vivo* [165]. These findings clearly implicated the therapeutic potential of targeting both XBP1 transcription and splicing. Interestingly, spliced XBP1 was found to suppress intestinal tumorigenesis [133]. Significant cross-talk also exists between these first two arms of the UPR, where the transcription of *XPB-1* gene is upregulated by ATF6 and *vice versa* [166-168]. However, unlike the function of first two arms of UPR, where the primary goal is to facilitate homeostasis, the third arm of the UPR is responsible for dictating the stress-adaptation and stress-susceptibility of cells [169-173]. Accumulating evidences suggest that this third arm is directly involved in UPR progression to ERS, and this metabolic switch primarily occurs via the critical regulation TRIB3 protein levels [48, 58, 64, 66, 88-91, 120, 134, 148, 160, 174]. Numerous studies have associated TRIB3 with stress-induced cellular dysfunctions and death. The ERS-induced pancreatic β -cell apoptosis via the NF- κ B pathway was shown to be regulated by TRIB3 [64, 88]. Furthermore, in high-fat-fed obese mice, skeletal muscle insulin resistance was clearly associated with increased TRIB3 expression [48]. The hypoxia-induced TRIB3 was clearly linked to decreased AKT levels and good prognosis in breast cancer [89] and colorectal cancer [134] patients.

In the crucial third arm of UPR, following dissociation from Grp78, the stress sensing domain of PERK is activated via both auto-phosphorylation and homo-dimerization [**Figure-2**]. Activated PERK then phosphorylates eIF2 α (eukaryotic translation initiation factor 2 alpha) which reduce ER protein load and enable homeostasis. However, although activated eIF2 α can inhibit global translation, a few proteins

continue to be synthesized due to their internal translation initiation sites (cap-independent). These UPR stimulated transcription factors, i.e. ATF4 and CHOP, are responsible for progression of UPR to ERS via augmenting TRIB3 gene expression [160, 175]. TRIB3 expression is primarily regulated at the level of transcription by concerted actions of two basic leucine zipper (bZIP) transcription factors, CHOP and ATF4 [95, 96, 111, 117, 128, 160, 175, 176]. Although the translation of ATF4 (activated transcription factor-4) initially increases expression of chaperone proteins and promote homeostasis, since ATF4 also promotes the synthesis of another transcription factor, CHOP (C/EBP homologous protein) [110, 160, 175] it is the most crucial factor in dictating TRIB3 expression. In addition, the balance of UPR and ERS can also be fine-tuned at this juncture via both a negative feedback loop to suppress ATF4 and CHOP expression and a cross-talk with other parallel signaling pathways that regulate the downstream effects [26, 66, 109, 137, 161, 170, 177, 178]. Indeed, TRIB3 can regulate both its own degradation [66, 74, 96, 117] as well as the degradation of both CHOP and ATF4 [57, 126, 177-179]. Thus, TRIB3 has been considered as both a target as well as a modulator of its own induction [Figure-3].

In summary, under conditions of transient or mild stress, although the coexpression of ATF4 and CHOP increases TRIB3 transcription, TRIB3 also blocks their function on the *TRIB3* gene via a negative feed-back loop. However, under prolonged ERS the accumulation of ATF4 and CHOP leads to the over-expression of TRIB3, and TRIB3 sequestration by other proteins and TRIB3-mediated suppression of AKT (as discussed below) suppresses homeostasis and facilitates cellular dysfunctions and apoptosis. Thus, the overexpression of TRIB3 trips the switch from survival to death.

V. TRIB3 regulates multiple stress response pathways

5.1. Cross-talks between PI3K/AKT/mTOR, autophagy and TRIB3. In addition to the UPR cascades initiated within the ER, cytosolic and mitochondrial proteins also provide parallel mechanisms to adjust intracellular stress in response to multiple exogenous stimuli [Figure-4]. One of these critical

interactions includes TRIB3-mediated targeting of the PI3K/AKT/mTOR and Autophagy cascades [180-185]. The PI3K/AKT/mTOR pathway is of crucial importance in regulating normal metabolic functions [67, 128, 183] and this pathway is often activated in aggressive cancer cells [26, 40, 170]. Briefly, PI3K (phosphatidylinositol 3-kinase) activation phosphorylates and activates AKT/PKB (protein kinase-B). The activated AKT initiates a number of downstream effectors such as CREB (cAMP response element binding protein) and mTOR (mammalian target of rapamycin). Interestingly, the first evidence of TRIB3 as a negative modulator of AKT and mTOR activity was provided by Du *et. al.*(2003) [123]. The serine/threonine protein kinase mTOR also plays a direct role in regulating cell survival via autophagy. Similar to the proteasomal degradation machinery, autophagy is another protein degradation system in the lysosome, and numerous studies demonstrate its involvement in pathophysiological processes [54, 56, 62, 137, 170, 181]. Similar to the three UPR cascades discussed above, both mTOR and autophagy pathways can also sense nutrient levels in cells, by integrating second messenger signaling from factors like insulin, growth factors, and amino acids. In 2010, Liu *et. al.* had shown that overexpression of TRIB3 in skeletal muscle cells of diabetic patients can reduce insulin-stimulated AKT activity [187] and a follow-up study by this same group corroborated the crucial role of TRIB3 in regulation of nutrient metabolism during both short-term fasting or glucose excess [49]. Indeed, the skeletal muscle is a major site of glucose disposal and one of the major characteristics of diabetes patients is reduced insulin sensitivity due to decreased glucose metabolism in skeletal muscles. Glucose metabolism has been directly linked to both inflammatory diseases and cancer via the 'Warburg effect' [50]. Chronic hypoxia alters cellular glucose metabolism so cells can adapt to the low oxygen by increasing HIF (hypoxia-inducible factor). Indeed, hypoxia induced activation of adipose tissue and endothelial cells are unified mechanisms for a variety of metabolic disorders [180]. Several regulators of glycolysis have also been identified as oncogene candidates, e.g. c-Myc, p53, HIF-1 α and Ras, and the interplay between glycolysis and oncogenic events

has been recently reviewed by Mikawa *et al.* (2015) [188]. Both hypoxia and PI3K, both regulators of TRIB3, have often been implicated in regulating glycolysis and the ‘Warburg effect’ in cancer cells [53]. Indeed, a direct link of TRIB3 to these metabolic pathways has been documented recently [52]. Schwarzer *et al.* (2006) showed that TRIB3 expression is selectively triggered in response to the lack of nutrients like amino acid and glucose [52]. Similarly, Okamoto *et al.* (2007) showed that TRIB3 is a suppressor of PI3K/AKT activity in conditions of fasting [186]. Thus, TRIB3 plays a direct role in regulating both PI3K/AKT/mTOR and autophagy.

5.2. Cross-talks between NF- κ B, MAPK and TRIB3. A number of investigators have provided evidence that both the ‘pseudokinase’ action can regulate the NF- κ B signaling pathway as well, and its MEK1 motif is well-known to negatively regulate the MAPK axis. The COP1 domain of TRIB3 can also assist in ubiquitin-mediated degradation of multiple factors involved in both of these signaling cascades [Figure-4]. Although previous investigations have suggested that TRIB3 is primarily regulated by ATF4 and CHOP [57, 126, 177], a number of studies also show that *TRIB3* gene is transcriptionally activated via the PKC (protein kinase-C) induced transcription factor NF- κ B (nuclear factor kappa of B-cells) as well as by several of the transcription factors activated via the MAPK pathway [64, 66, 68, 82, 84, 96, 101, 108, 117, 128, 140, 145, 149]. The MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) also communicates mitogenic signals from receptors, e.g. epidermal growth factor receptor (EGFR). Inflammatory cytokines, e.g. IL-1 β , IL-3 and IL-6, are known to increase TRIB3 expression. On the other hand, anti-inflammatory agents like dexamethasone and cAMP, reduced TRIB3 expression via the transcription factors CREB and FOXO-1 (Forkhead box protein O1) [117, 189, 190]. Several studies have also shown that amino acid excess (or depletion) can similarly increase TRIB3 expression [191, 192]. Indeed, both CHOP and ATF4 are amino acid responsive genes and contain AARE (amino acid response elements) sequences in their promoter regions [191]. Carraro *et al.* (2010) also showed

that the binding of ATF4 to these AARE sequences is crucial in the transcriptional activation of TRIB3 [192]. These investigators documented that a leucine deficient diet leads to the induction of TRIB3. Thus, multiple cellular stressors that activate the PKC and MAPK pathways can regulate TRIB3 function by controlling both TRIB3 gene expression and protein stability, and may be a potential target against both metabolic syndrome and cancer. Indeed, our previous published study in an aggressive prostate cancer cell line, C4-2B showed that combined exposure to the anti-HIV drug Nelfinavir, which induces UPR and autophagy [193, 194] and the phytochemical Curcumin, which suppresses NF- κ B [195, 196] can subvert UPR towards ERS, and significantly increased apoptotic cell death [110]. Interestingly, although combined exposure to these two agents increased eIF2 α and ATF4 expression in both transformed cells (C4-2B) and normal cells (RWPE-1), simultaneous activation of UPR by Nelfinavir and suppression of NF- κ B by Curcumin induced the death sensors CHOP and TRIB3 only in C4-2B cells, but not in RWPE-1 cells [110]. Similarly, we have recently shown that the Nelfinavir-mediated ERS can increase TRIB3 levels in an aggressive multidrug resistant (MDR) breast cancer line (MCF-7/Dox). Coexposure to Nelfinavir resulted in significant chemosensitization of MCF-7/Dox cells to the anticancer agent, Doxorubicin. Profound increases in *in vitro* cell death and decreased tumor growth in *in vivo* tumor xenografts were documented in these studies (*Accepted Manuscript* included in this issue). Therefore, strategies to induce TRIB3 in cancer cells via targeting the cross-talks between the UPR cascade with both the NF- κ B and MAPK cascades may have significant potential as promising anti-cancer treatment approaches.

VI. TRIB3 association with metabolic dysfunctions

6.1. TRIB3 suppresses insulin signaling and glycogen storage. Metabolic syndrome results from a failure of uptake, storage and utilization of excess glucose in the circulation due to dysregulated insulin receptor (IR) function, which is primarily responsible for systemic insulin resistance [197] **[Figure-5]**.

Briefly, the insulin receptor (IR) is composed of two α and β subunits consisting of extracellular domains, transmembrane and cytoplasmic domains. Insulin binds to the extracellular subunits of IR and prompts a conformational change resulting in auto-phosphorylation of tyrosine residues. Phosphorylated IR is then recognized by insulin receptor substrate (IRS) family members, the phosphor-tyrosine binding (PTB) adapter proteins. Phosphorylated IRS then activates the regulatory subunit of PI3K (p85) and the catalytic subunit of PI3K (p110) then phosphorylates phosphatidylinositol bis-bisphosphate (PIP₂) and results in the formation of PIP₃. An important downstream effector of PIP₃ is the transcription factor AKT (PKB) which then activates multiple downstream cellular processes such as glucose metabolism, cell proliferation, cell migration and apoptosis [198, 199]. Insulin signaling triggers the uptake of glucose in the liver, adipose tissue and muscles; where it is stored as glycogen [200, 201]. Therefore, one of the most important effects of activated AKT (phosphorylated at both Serine⁴⁷³ and Threonine³⁰⁸ residues) is the glycogen synthesis cascade. Activated AKT phosphorylates and inactivates GSK3 (glycogen synthase kinase 3) which inhibits the enzyme glycogen synthase. Indeed, numerous studies have shown that glycogen synthesis is blocked by high levels of ERS [202, 203] and increased TRIB3 expression [123, 204]. AKT inactivation by TRIB3 dysregulates hepatic glucose production and thus further promotes insulin resistance. Importantly, a point mutation in TRIB3 (R84 variant) has often been associated with impaired glycogen synthesis [205]. The AKT-suppressive effects of TRIB3 can also block insulin-induced NO release from endothelial cells and suppress cGMP production and relaxation of the underlying smooth muscle cells [85, 98, 115, 199, 206]. Interestingly, the same TRIB3 polymorphism has been associated with decreased NO production, as well [205]. Both the PI3K/AKT and the MEK/ERK signaling pathways are well established in insulin regulation of smooth muscle cells proliferation [199] and the silencing of TRIB3 was able to suppress atherosclerosis and stabilize plaques in the diabetic mice [115]. Another important role of insulin is in the stimulation of glucose uptake via the membrane

translocation of glucose transporter, GLUT4. Over-expression of TRIB3 in skeletal muscle cells can block GLUT4 translocation and suppress insulin-stimulated glucose uptake [187]. Indeed, it is worth mentioning that the endothelial dysfunctions and dyslipidemia observed with the clinically approved HIV-1 protease inhibitors like Nelfinavir has also been linked to the inhibition of both proteasome activity and glucose transport, both by us [207, 208] and others [209]. Thus, TRIB3 is linked to the ART (antiretroviral therapy) associated metabolic dysfunctions such as increased endothelial dysfunction, atherosclerosis and lipodystrophy, as well.

6.1.1. *TRIB3 overexpression in insulin resistance and diabetes.* It is well-known that diabetes is manifested due to decreased insulin-glucose homeostasis in pancreatic islets, vascular endothelial and smooth muscle cells, in both adipocytes and stem cell progenitors in the adipose depots [2, 37, 47]. Indeed, TRIB3 has been associated with all of these cellular dysfunctions [48, 66, 120]. Since TRIB3 is also ubiquitously expressed in liver, heart, kidneys, lung, skin, small intestines and stomach, it may be responsible for suppressed insulin signaling in multiple other tissues, as well. Amplified UPR and ERS are seen in both liver and adipose tissues of genetically obese (ob/ob) and diet-induced obese mice [210, 211]. Increased levels of Grp78, phospho-eIF2 α , spliced XBP1 mRNA, and CHOP proteins were observed in pancreatic islets from these mice, and correlated well with the severity of their insulin resistance [211]. Similarly, mice lacking XBP1 showed chronic hyperglycemia and increased β -cell loss and clearly implicated a protective role of XBP1 in insulin resistance [164, 167]. The UPR transducers, Grp78, XBP1s, phospho-eIF2 α and phospho-JNK were upregulated in both liver and adipocytes from insulin-resistant patients, as well [212]. Thus, although all three UPR pathways serve important physiologic roles in normal glucose homeostasis, prolonged UPR and the pathophysiologic effects of metabolic diseases are primarily exacerbated due to increased TRIB3 levels via third arm of the UPR (PERK-eIF1 α) [64, 88, 95, 115, 121]. Interestingly however, a recent clinical finding by Boden *et al.*

(2014) suggested that insulin resistance is actually associated with diminished ERS responses in adipose tissue of healthy and diabetic subjects [213]. In this respect, it has been observed that the ATF6 branch of the UPR may be beneficial in augmenting the transcription of gluconeogenic genes and lowers blood glucose levels in ob/ob mice [167, 214]. Since TRIB3 can regulate the ATF6 arm via suppressing the PERK-eIF2a-ATF4 axis [174], precise regulation of TRIB3 expression and its downstream effects may be utterly vital in the progression of insulin resistance and diabetes.

6.1.2. *TRIB3 is associated with atherosclerotic plaque rupture.* A number of recent studies have provided evidence that TRIB3 plays a direct role in atherosclerosis progression. Wang *et al.* (2012) showed that TRIB3 knockdown in a diabetic mouse model can significantly decrease blood glucose and increase liver glycogen levels [115]. In this study, TRIB3 was also shown to play a direct role in destabilizing atherosclerotic plaques [115]. Phenotypic characteristics of atherosclerotic plaque destabilization, such as fibrous cap thickness, collagen content and plaque cap-to-core ratio, were all altered by overexpression of TRIB3. Additionally, TRIB3 silencing decreased the number and size of aortic plaques. Berisha *et al.* (2013) carried out transcriptome analysis of genes in two strains of mice with atherosclerosis susceptibility [215]. The response to cholesterol-loading of macrophages (foam cells) from DBA/2 and ApoE(-/-) mice were tested by gene expression profiling, which identified three genes known to participate in the ERS stress response, Ddit3 (CHOP), ATF4 and TRIB3 [215]. Further corroborative evidence on the role of different TRIB isoforms, changes in their expression and subcellular localization in atherosclerotic tissues/cells and development of TRIB3 targeting agents may provide new and highly promising avenues to suppress both atherosclerosis and stroke.

6.1.3. *TRIB3 is associated with β -cell dysfunction and death.* Type-1 Diabetes Mellitus (T1DM) is manifested due to the pancreas not producing enough insulin and progressive β -cell loss. Recent evidences clearly implicate a role for TRIB3 in pancreatic β -cell dysfunctions [88, 179]. Qian *et*.

al. (2008) carried out studies in Goto-kakizaki (GK) rats, a model for T1DM with progressive loss of β -cell function, and showed higher increases in TRIB3 in the hyperglycemic rats, as compared to normoglycemic rats [88]. Furthermore, these investigators demonstrated the deleterious effects of TRIB3-mediated upregulation of caspase-3 activity and apoptosis, which were precipitated under high glucose concentrations. Similar apoptotic death of cardiac myocytes by TRIB3 was also observed in a rat model of cardiomyopathy by Ti *et al.* (2011) [216]. Importantly, strategies towards TRIB3 gene silencing were able to alleviate diabetic cardiomyopathy in these rats. Zhang *et al.* (2013) showed increased TRIB3 expression in the skeletal muscle of diabetic rats within 10 days of hyperglycemia [217]. Interestingly, glucose-stimulated TRIB3 expression was dependent on the nutrient-sensing carbohydrate synthesis pathway, and azaserine, an inhibitor of the hexosamine biosynthetic pathway, was able to suppress TRIB3 expression in this model [217]. Thus, strategies that suppress the deleterious effects of TRIB3 and its interactions with crucial survival pathways in islet cells may be beneficial.

The above observations in multiple diabetes-associated diseases have clearly incriminated TRIB3 as a crucial etiologic agent, and implicated its potential as both a biomarker and pharmacological target. Below, we discuss findings that associate TRIB3 polymorphisms with aggressive disease phenotypes.

6.1.4. The TRIB3 Q84R polymorphism in diabetes and atherosclerosis. An intriguing observation has been that the Q84R (rs2295490) genetic polymorphism, which codes for a gain-of-function variant of TRIB3, can increase the risk of diabetes and atherosclerosis development. Interestingly, the variant with Arginine at amino acid 84 (R84) is a stronger inhibitor of insulin-mediated AKT activation as compared with the more frequent Glutamine (Q84) variant. A number of recent investigations have indeed associated this specific TRIB3 genotype with the metabolic syndrome [217-223]. This polymorphism was first linked to impaired insulin-mediated NO production in human endothelial cells [205] and subsequent findings have linked this variant with both cardiovascular risk

[218] and early-onset diabetes in Caucasians [219]. Several studies have also suggested that this TRIB3 polymorphism is a risk factor for carotid atherosclerosis [220, 221]. Interestingly, although the wild-type TRIB3 is known to suppress MAPK signaling [85, 107] the Q84R TRIB3 variant causes enhanced MAPK function in endothelial cells, and this function was connected to increased intima-media thickness [222]. This further underscored the importance of TRIB3 function, concentration, context dependency and polymorphisms [49, 90]. In a recent review, Prudente *et al.* (2015) illustrated that the Q84R polymorphism is relatively common and is frequently associated with abnormal insulin signaling, endothelial dysfunction, pro-atherogenic phenotypes, and other related metabolic abnormalities [223]. Findings also implied that other TRIB3 polymorphisms may be present in different ethnic population, and may alter the effects of distinct TRIB3 functional domains that drastically alter its multimodal effects in regulating both homeostasis and disease. Interestingly however, despite the clear associations between TRIB3 and numerous other diabetes-associated diseases such as hyper-homocysteinemia (HHcy) [113, 204], non-alcoholic fatty liver disease (NAFLD) [223-225], diabetic nephropathy (DN) [226-228] and visceral obesity [224, 229-231]; this Q84R variant is not currently being used as a prognostic indicator of disease progression in these diabetes-associated diseases. Furthermore, despite the increased linkages between diabetes and cancer [7, 9, 11-13] and ample evidences documenting the role of TRIB3 as a common nexus [49, 89, 90, 134], the ability of Q84R or other possible TRIB3 variants in predicting tumor phenotype, especially in patients with diabetes, has not been thoroughly investigated.

6.2. TRIB3 is associated with numerous diabetes-associated diseases.

6.2.1. Hyper-homocysteinemia. Hyper-homocysteinemia (HHcy) occurs due to elevated levels of circulating homocysteine and is often associated with atherosclerosis [232, 233]. Similar to hyperglycemic conditions, high concentrations of homocysteine can induce TRIB3 expression [113, 204]. Interestingly, unlike the role of PKC-induced TRIB3 in inflammatory stress, the HHcy-mediated

induction in TRIB3 was linked to a PKA-dependent pathway [113]. The transcription factors CREB (cAMP responsive element binding protein), not ATF4, CHOP or NF- κ B, was found to activate TRIB3 under HHcy conditions [113]. In addition, in contrast to previous findings on TRIB3 mediated suppression in cell proliferation, the HHcy-induced augmented TRIB3 levels resulted in smooth muscle hypertrophy [204]. However, similar to other systems, TRIB3 silencing had a protective role and decreased hypertrophy. Furthermore, increased TRIB3 levels in HHcy patients was independent of the ERS transducers PERK or eIF2 α , implicating alternate stress response pathways activated in HHcy that may enhance TRIB3 expression.

6.2.2. Non-alcoholic fatty liver disease. Insulin resistance also plays an important role in non-alcoholic fatty liver disease (NAFLD) a major cause of cryptogenic cirrhosis [234] and liver cancer [235]. Indeed, TRIB3 has been implicated in both initiation and development of NAFLD primarily via saturated fatty acid mediated insulin resistance [224]. In a rat model of NAFLD, Wang *et al.* (2009) showed that mild to moderate hepatic steatosis did not produce increases in TRIB3; however, both TRIB3 mRNA expression and protein levels were significantly higher in rats with fatty hepatitis [225]. Prudente *et al.* (2015) had also postulated that the Q84R polymorphism may be linked to these metabolic alterations, as well [223]. Thus, TRIB3 may be utilized as an early biomarker of cryptogenic cirrhosis and therapeutic targeting of TRIB3 may be a new strategy against NAFLD, especially in patients with severe steatosis.

6.2.3. Diabetic nephropathy. Both kidney dysfunctions and nephropathy are observed at a high percentage of patients with type-2 diabetes [236, 237]. Morse *et al.* (2010) documented that TRIB3 plays an important role in diabetic nephropathy in a mouse model [238]. In addition, TRIB3 was up-regulated in kidneys of rats with type-1 diabetes [226]. TRIB3 overexpression also resulted in significant apoptosis in renal tubular cells [227]. Increased oxidative stress and ER-stress have been associated with severe loss of podocytes and the resultant defective kidney function under hyperglycemic conditions. Although

high glucose did not increase TRIB3 expression in normal podocytes, proliferating podocytes showed significant upregulation of TRIB3. This was especially evident when they were exposed to hyperglycemic conditions [227]. The induction of ROS (reactive oxygen species) by H₂O₂ (hydrogen peroxide) further augmented TRIB3 expression and nephropathy in activated podocytes. The chemokine MCP1 (macrophage chemotactic protein-1) contributes to inflammatory injury associated with nephropathy. Interestingly, it has been observed that MCP1 is inhibited by TRIB3 [238]. This suggested that the anti-inflammatory effects of TRIB3 in the kidney may be a protective mechanism in diabetic nephropathy. A recent study by Zhang *et al.* (2015) showed that the Q84R polymorphism is indeed associated with diabetic nephropathy in Chinese patients [228]. Although kidney dysfunctions are observed at a significantly higher rate in African American population, the association of this Q84R polymorphism may implicate new directions in understanding this health disparity and guide better therapeutic options.

VII. Linking TRIB3 expression in obesity and cancer

7.1. TRIB3 expression in adipocyte differentiation and visceral obesity. Increase in visceral adiposity is often accompanied with low grade inflammation and increases the aggressive behavior of neoplastic cells [7, 11, 14, 20, 30, 37, 152, 224]. Adipose cells behave as the inflammatory source in systemic inflammation, and hence, numerous studies have recently addressed the commonalities between obesity and cancer [17, 22, 122, 210]. It is well accepted that the balance between lipogenesis and lipolysis is linked to increase in BMI, and insulin signaling plays a critical role in both differentiation of pre-adipocytes and fatty acid release from lipid-laden adipocytes. Recent advances in dissecting the molecular mechanisms involved in adipogenesis and their lipid metabolism clearly indicate that the UPR and ERS pathways play central roles [152, 224, 238, 239]. Stress in the adipocytes can occur due to nutrient and energy overload, increased demand for protein synthesis and local glucose deprivation. Scheuner *et al.* (2005) showed that both UPR function and the secretion capacity of ER are augmented

following continuous increases in blood glucose levels [240]. Mihai *et al.* (2015) recently reported that the pre-existence of mild ERS can predispose adipocytes to an exacerbated response, especially when they are exposed to inflammatory cytokines like IL-1 β or TNF- α [241]. However, despite these associative evidences, obesity has not been linked to any specific UPR pathway or to any distinct UPR transducers, and the importance of TRIB3 has been largely overlooked in the adipose tissue. A recent *in vitro* study in both 3T3-L1 cells and in genetically engineered obese mice clearly depicted that impaired eIF2 α phosphorylation enhances adipocyte differentiation [242]. In addition, forced production of CHOP, a downstream target of eIF2 α and an upstream regulator of TRIB3, was able to potently inhibit insulin-induced adipogenesis [242]. In the Akita mouse model of diabetes, the obesity and hyperglycemia-induced oxidative stress was accompanied by increased CHOP expression, and the deletion of CHOP was able to reduce obesity, β -cell dysfunction and systemic inflammation [243, 244]. However, TRIB3 levels were not measured in these mice. The above studies also showed that stress signaling via eIF2 α and CHOP, but not IRE1 α , suppresses adipogenesis and limits the expansion of fat mass *in vivo*. This implicated that several UPR transducers are associated with obesity-induced dysfunctions. In addition to the role of insulin-induced glucose uptake via GLUT4 during adipogenic differentiation, adipose stem cells also utilize insulin-stimulated p-AKT for increased proliferation and survival. Appropriate adipogenic differentiation also requires temporal increases in lineage specific transcription factors such as C/EBP α and PPAR γ . Due to its suppressive actions on both MAPK signaling [85, 107, 222] and on increased proteasomal degradation of C/EBP α and PPAR γ [80, 143, 145], it is likely that TRIB3 plays a direct role in regulating obesity [242, 245]. Studies have also demonstrated a crucial role of MEK/ERK signaling in regulating both C/EBP β and PPAR γ expression during adipogenesis [245]. Indeed, several recent studies have documented that TRIB3 overexpression is a potent negative regulator of adipogenesis [246] and TRIB3 interactions with PPAR γ is involved in inhibiting stem cell differentiation [143]. Under

homeostatic conditions, TRIB3 was indeed found to be downregulated during the clonal expansion phase in mouse 3T3-L1 pre-adipocytes. However, adipogenesis decreased and lipolysis increased when TRIB3 is overexpressed. Similarly, inhibition of adipogenesis is also accompanied by a TRIB3-mediated decrease in phosphorylation of C/EBP β [74, 77]. Studies have shown that TRIB3 can physically interact with C/EBP β at the repressor domain-1, thereby inhibiting its DNA binding ability and transactivation function. Both MCP-1 and the MCP-1-induced protein (MCPIP) are known inducers of adipogenesis. Thus, similar to studies in podocytes, where TRIB3 inhibits the chemokine MIP-1 [238], TRIB3 inhibited both MCP-1 and MCPIP in adipocyte progenitors [247]. However, a precise regulation of its effects via cross-talk with the MAPK pathway may also be important [245]. A few recent studies have also implied a direct role of TRIB3 polymorphisms (especially Q84R) in susceptibility towards increased adiposity and adipokine production [223, 231].

Studies have documented a clear link between obesity and aggressive phenotype of different cancers [9-11]. The potent anti-tumor effects of metformin may function via a TRIB3 regulated mechanism [17, 22, 210]. The newly developed antitumor drug ABTL0812 is known to upregulate TRIB3 [56]. Therefore, targeting of TRIB3 may be a new direction in both insulin resistance and cancer research. Furthermore, since the immunomodulatory effects of adipose tissue is often associated with tumor growth and several studies show an emerging role of TRIB3 on immune cell functions [117-119]. Thus, effects of TRIB3 on cancer immune surveillance may be an unexplored connection between obesity and aggressive cancers.

7.2. Potential of targeting TRIB3 in aggressive cancers. Tumor cells are influenced by continuous paracrine signals from the surrounding microenvironments, and their malignant transformation enable a higher threshold stress-adaptation. Indeed, because of their high proliferation rates, cancer cells require increased protein assembly capacity in the ER [133, 137, 248]. Hence, the combined targeting of

UPR/ERS responses and the parallel survival mechanisms in aggressive cancer cells is becoming a promising therapeutic approach [110, 175, 203, 249]. In a recent study, Hou *et al.* (2015) showed that PERK silencing inhibits growth of aggressive glioma cells by blocking phospho-AKT levels [250]. A constitutively active PI3K/AKT/mTOR axis is a key driver in carcinogenesis and Everolimus, an mTOR inhibitor, is currently approved for the treatment of HER2-negative breast cancer [251]. Due to its potent AKT/mTOR suppressive effect, TRIB3 overexpression may similarly be a potent anti-tumor strategy. In hypoxic tumor microenvironments, autophagy is also activated to counteract the effects of chronic inflammation and oxidative stress [252]. Autophagy also facilitates the resistance of tumor cells to anticancer agents, and the suppression of autophagy has been beneficial in cancer treatments [26, 62, 109, 253]. In a recent study, cannabinoids were shown to induce autophagy-mediated cell death in human glioma cells by stimulating the ERS pathway [137]. This antitumor effect of cannabinoids was also directly linked to significant TRIB3 induction [161]. In mouse models of hodgkin's lymphoma, combined exposure to the AKT-inhibitor perifosine and the RAS/RAF-inhibitor sorafenib could increase tumor cell death [136]. TRIB3 was identified as the main mediator of the antitumor activity of perifosine/sorafenib combination [136]. Thus, the targeting of multiple survival pathways by TRIB3 may be very effective and may also delay drug resistance.

Poor vascularization of tumors, which leads to hypoxic stress increases TRIB3 gene expression [81, 89]. Hypoxia negatively impacts tumor response to therapy by inducing eIF2 α phosphorylation and hypoxia-mediated increase in angiogenesis is essential for tumor growth [50, 254]. Indeed, a highly promising antitumor strategy has been directed towards the suppression of HIF-1 α [255]. In breast cancer patients, Wennemer *et al.* (2011) showed that increased TRIB3 expression is directly associated with a better prognosis in metastatic cancers [89]. However, unlike the previous studies with colorectal cancer specimen [134], TRIB3 protein levels did not correlate well with TRIB3 mRNA levels in breast cancer

cells [89]. Intriguingly, the high TRIB3 protein expressing tumors were found to be hypoxia sensitive and the low TRIB3 expressing tumors were more hypoxia tolerant [58, 89, 96]. Furthermore, the siRNA mediated knockdown of TRIB3 improved hypoxia survival of breast cancer cells. These findings implicated a TRIB3 targeting strategy to sensitize tumor cells to hypoxic microenvironments. Simultaneous targeting of both aggressive tumor cells and the tumor-associated endothelium may also be a highly viable approach in TRIB3 directed antitumor therapy.

Elevated levels of Grp78 correlate well with higher pathologic grade, recurrence rate, and poor survival in cancer patients [256]. Increased expression of Grp78 in visceral adipocytes also predicts endometrial cancer progression and decreased patient survival [257]. This provides a crucial link between obesity and cancer and implicates TRIB3 as the crucial metabolic switch and a viable tumor biomarker [7, 14]. Although the overexpression of other UPR transducers such as XBP1 and ATF6 have been shown in human cancers including breast and hepatocellular carcinomas [258, 259] the PERK-eIF2 α axis, which regulates TRIB3 expression, seems to play a dominant role in tumor progression [250]. In a transgenic mouse model overexpressing spliced XBP1, neoplastic transformation of plasma cells and development of multiple myeloma were also clearly observed [260]. Furthermore, an IRE1 α endonuclease specific inhibitor, which regulates XBP1 splicing, was shown to have potent cytotoxic activity in cancer cell [261]. Tumors undergoing nutrient deprivation can activate multiple UPR transducers, and both IRE1 α and eIF2 α can increase ATF4 and CHOP to drive TRIB3 gene expression and death. Thus, studies are focusing on TRIB3 as a biomarker for aggressive tumors and potentially as a marker of therapy outcome.

TRIB3 was found to be overexpressed in lung, colorectal, breast and ovarian tumor models [58, 107, 134, 135]. In addition to the PI3K/AKT/mTOR cascade, TRIB3 also regulates important cancer signaling pathways such as MAPK-ERK, TGF β and JAG1/Notch pathways. Abnormal expression of TRIB3 is a prognostic indicator of morbidity and mortality in colorectal cancer [134]. Increase in TRIB3

expression also correlated with poor prognosis in breast cancer patients [58]. Interestingly however, TRIB3 knockdown induced apoptosis in human lung cancer cells via the regulation of Notch 1 expression, which is a crucial regulator of epithelial-mesenchymal transition (EMT) phenotype [135]. Activated Notch signaling and growth promoting effects of Notch target genes are often seen in aggressive cancer cells, and thus, TRIB3 may play a vital role in tumor progression and metastasis. TGF- β 1 also plays a very important role in tumor progression, and increased TGF- β 1 levels can promote tumor invasion. In aggressive breast cancer cells, a high throughput kinase inhibitor screen revealed that both TRIB3 and TGF β are Notch regulators [134]. Indeed, TRIB3 plays a crucial role in regulating TGF- β signaling by physically interacting with SMAD3, a downstream modulator of TGF- β [99, 107]. Hua *et. al.* (2011) showed that TRIB3 can enhance TGF- β 1/SMAD mediated transcriptional activity and tumor cell survival [99]. These investigators showed that TRIB3 maintains the mesenchymal status of HepG2 cells and regulated TGF- β 1-induced EMT of hepatocellular carcinoma cells. Zhou *et. al.* (2013) showed that TRIB3 expression is upregulated in non-small cell lung cancer (NSCLC) and provided a subsequent correlation with EMT phenotype such as tumor metastasis, disease recurrence and poor patient survival [135]. A prognostic study using patient tumor specimens also revealed that the overall survival rate was significantly lower in patients showing high TRIB3 expression. Miyoshi *et. al.* (2009) also showed an overexpression of TRIB3 in colorectal cancer and both TRIB3 mRNA and protein in tumor samples were associated with poor prognosis [134]. *In vivo* studies showed that siRNA mediated inhibition of TRIB3 significantly decreases cell growth in seven different cancer cell lines. Therefore, investigations in different tumor models suggest that TRIB3 may function as both an oncogene and a tumor suppressor.

Interestingly, in contrast to the studies showing that UPR is activated in different human tumors, a recent report suggests that UPR is actually down-regulated in prostate cancer [263]. The role of UPR and ERS in cancer cells may thus be more complex than previously envisioned, and may be dependent upon

both tumor genotype and phenotype, as well as on the stressors within the tumor microenvironments. Further studies looking at TRIB3 expression in prostate cancer specimen may be of significant benefit in recognizing indolent vs. aggressive disease. Furthermore, since steroid hormone action is critically affected by insulin resistance and since TRIB3 is known to alter insulin signaling, detailed tumor pathological analysis of TRIB3 expression and its subcellular localization may provide an early biomarker of hormone resistance phenotype [110, 264, 265].

A TRIB3 enhancing antitumor agent (ABTL0812) has recently entered clinical trial and was shown to inhibit the Akt/mTORC1 pathway [56]. Another possible strategy to exploit the actions of TRIB3 in tumors may be to target the upstream transducers of the ERS pathway. Indeed, chronic low levels of ERS may increase TRIB3 expression and act in a prosurvival capacity, and it would be necessary to induce severe ERS that leads to TRIB3 overexpression. This strategy will be able to subvert the survival mechanisms towards a sustained ERS in tumor cells, which will activate TRIB3 and the mitochondrial pro-apoptotic caspases [110]. Using cells cultured from uterine leiomyoma, Xu *et al.* (2007) showed that the induction of severe ERS by using Asoprisnil can increase TRIB3 expression in a CHOP-dependent manner, which significantly induced apoptosis [266]. Suppression of CHOP by RNA interference was able to reduce this Asoprisnil-induced TRIB3 and decreased leiomyoma cell death [266]. We have also shown that, by subverting ERS towards apoptosis by Nelfinavir (autophagy inducer) and Curcumin (NF- κ B suppressor) coexposure augments Docetaxel efficacy in castration resistant prostate cancer (CRPC) cells [110]. Although the basal level of TRIB3 was low in aggressive CRPC cells (C4-2B), its expression was rapidly increased following the induction of severe ERS by Nelfinavir and Curcumin coexposure and significantly increased apoptosis even at subtoxic concentrations of Docetaxel [110]. Interestingly, we also observed that TRIB3 protein induction was evident within 6 hours post drug treatment; which was significantly earlier than the observed increases in Grp78. These findings suggested

that an alternate downstream UPR effector may regulate TRIB3. Indeed, our drug combination studies emphasized that simultaneous targeting of Autophagy and NF- κ B pathways enable a robust and rapid increase in TRIB3, specifically in aggressive cancer cells [148, 193, 196]. Although Nelfinavir and Curcumin combination rapidly increased both ATF4 and CHOP expression in C4-2B cells, studies using the normal prostate cell line, RWPE-1, showed that only CHOP expression was increased and not ATF4. This further confirmed an earlier finding by Bromati *et al.* (2011) that CHOP and ATF4 coexpression is vital for significant increases in TRIB3 expression, which is needed to suppress AKT and induce cell death [160]. Interestingly, ionizing radiation mediated activation of PERK/eIF2 α /ATF4 signaling was similarly found to act via an ERS-independent pathway in human vascular endothelial cells [174]. Thus, a thorough understanding of pathways that regulate TRIB3 induction and precise regulation of its downstream functions will provide novel anticancer and therapy-sensitization approaches.

VIII. Perspectives

Multimodal actions of TRIB3 in regulating cell proliferation, differentiation and metabolism, implicates its role in sensing, integrating, and responding to diverse signals that dictate homeostasis. Current evidences clearly show that this TRIB3 balance is tipped in both metabolic diseases and cancer [Figure-6]. Indeed, more and more evidences correlate that TRIB3 serves as a switch between multiple cellular signaling pathways, and its effects may ultimately be dependent upon the duration and magnitude of the stress stimulus. However, many unanswered questions remain as to the mechanisms and consequences of TRIB3 involved processes that ultimately propel cells towards dysfunctions and death. The identification of TRIB3 as a crucial factor at the juncture of health, metabolic diseases and cancer, and the utility of TRIB3 targeted agents against these diseases will be important for the translation of novel therapeutic approaches. The promiscuous nature of TRIB3 enables it to bind various proteins and

regulate their biological functions, and thus, TRIB3 plays an important role in insulin signaling in both normal and cancerous cells. The regulatory effects of TRIB3 on AKT and MAPK signaling and its role in ubiquitination and proteasomal degradation of transcription factors such as ATF4, CHOP, NF- κ B, C/EBP α & PPAR γ , further demonstrate its role at the nexus of proliferation and differentiation in both normal and dysfunctional cells. Indeed, a better understanding of how the associations of TRIB3 with different cellular factors are fine-tuned to enable its crucial functions will be of great importance. Since TRIB3 regulates apoptotic signaling in tumor cells, recent evidences also suggest its potential as a target in aggressive tumors. Furthermore, emerging evidences also indicate that the other two family members, TRIB1 and TRIB2, may regulate multiple cellular pathways, as well. Indeed, the cross-talks between these mammalian TRIB proteins will add further complexity in their ability to regulate cellular functions, but at the same time, this knowledge may also help develop more precise therapeutic targets. The association of TRIB3 Q84R polymorphism with increased risk of diabetes and atherosclerosis also point out its potential as a biomarker in metabolic diseases. The existence of other variants in TRIB proteins and delineating their relationship to other diseases will thus be of significant clinical benefit, as well. The ability of TRIB variants in predicting aggressive tumor phenotype needs to be addressed, especially in regards to the racial disparity observed in many of the aggressive cancers such as prostate and breast cancers. Interestingly, despite decades of research with TRIB3, sex dependent differences in TRIB3 expression and function is still an uncharted territory, and findings on the regulation of TRIBs by steroid hormones need to be more thoroughly addressed. Last but not the least, although the ageing process is clearly associated with increased risk of metabolic diseases and cancer, changes in TRIB3 expression in the elderly has not been previously documented. During the past decade, there have been compelling evidences on the role of TRIB3 in metabolic diseases and aggressive cancers. Extensive research on this field has also illuminated our understanding of how differences in the ability of both

normal cells and cancer cells to sense and cope with environmental stress can be ameliorated or exploited for new therapy development. In this respect, the new discovery of a TRIB3 activating agent ABTL0812 and future evidence of its antitumor efficacy in clinical trials may provide an impetus towards TRIB3 targeted agents to suppress metabolic diseases, as well. Most importantly, more TRIB3 centered research may facilitate novel strategies to disrupt the crucial link between metabolic diseases and cancer.

Acknowledgements: This work was supported by grants from the Department of Defense (#PC080811) and funds from the Louisiana Cancer Research Consortium (LCRC) to DM.

IX. References

- 1: Samper-Ternent R, Al Snih S. Obesity in Older Adults: Epidemiology and Implications for Disability and Disease. *Rev Clin Gerontol*. 2012;22(1):10-34.
- 2: Zimmet PZ, Magliano DJ, Herman WH, Shaw JE. Diabetes: a 21st century challenge. *Lancet Diabetes Endocrinol*. 2014;2(1):56-64.
- 3: O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes Rev*. 2015;16(1):1-12.
- 4: Diaz-Valencia PA, Bougnères P, Valleron AJ. Global epidemiology of type 1 diabetes in young adults and adults: a systematic review. *BMC Public Health*. 2015;15:255-67.
- 5: Mansour M. The roles of peroxisome proliferator-activated receptors in the metabolic syndrome. *Prog Mol Biol Transl Sci*. 2014;121:217-66.
- 6: Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. *Rev Endocr Metab Disord*. 2013;14(1):5-12.
- 7: Rose DP, Gracheck PJ, Vona-Davis L. The Interactions of Obesity, Inflammation and Insulin Resistance in Breast Cancer. *Cancers (Basel)*. 2015;7(4):2147-68.
- 8: Cantiello F, Cicione A, Salonia A, Autorino R, De Nunzio C, Briganti A, Gandaglia G, Dell'Oglio P, Capogrosso P, Damiano R. Association between metabolic syndrome, obesity, diabetes mellitus and oncological outcomes of bladder cancer: a systematic review. *Int J Urol*. 2015;22(1):22-32.
- 9: Faulds MH, Dahlman-Wright K. Metabolic diseases and cancer risk. *Curr Opin Oncol*. 2012;24(1):58-61.
- 10: Wang T, Ning G, Bloomgarden Z. Diabetes and cancer relationships. *J Diabetes*. 2013;5(4):378-90.
- 11: Gallagher EJ, LeRoith D. Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality. *Physiol Rev*. 2015;95(3):727-48.
- 12: Steenland K, Nowlin S, Palu S. Cancer incidence in the National Health and Nutrition Survey I. Follow-up data: diabetes, cholesterol, pulse and physical activity. *Cancer Epidemiol Biomarkers Prev*. 1995;4(8):807-11.
- 13: Calle EE, Rodriguez C, Jacobs EJ, Almon ML, Chao A, McCullough ML, Feigelson HS, Thun MJ. The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design, and baseline characteristics. *Cancer*. 2002;94(9):2490-501.
- 14: Calle EE, Thun MJ. Obesity and cancer. *Oncogene*. 2004 Aug 23;23(38):6365-78.

- 15: Ge Z, Ben Q, Qian J, Wang Y, Li Y. Diabetes mellitus and risk of gastric cancer: a systematic review and meta-analysis of observational studies. *Eur J Gastroenterol Hepatol*. 2011;23(12):1127-35.
- 16: Hope C, Robertshaw A, Cheung KL, Idris I, English E. Relationship between HbA(1c) and cancer in people with or without diabetes: a systematic review. *Diabet Med*. 2015. doi: 10.1111/dme.13031.
- 17: Casadei Gardini A, Marisi G, Scarpi E, Scartozzi M, Faloppi L, Silvestris N, Masi G, Vivaldi C, Brunetti O, Tamberi S, Foschi FG, Tamburini E, Tenti E, Ricca Rosellini S, Ulivi P, Cascinu S, Nanni O, Frassinetti GL. Effects of metformin on clinical outcome in diabetic patients with advanced HCC receiving sorafenib. *Expert Opin Pharmacother*. 2015;16(18):2719-25.
- 18: Mendonça F, Soares R. Obesity and cancer phenotype: Is angiogenesis a missed link? *Life Sci*. 2015;139:16-23.
- 19: Andò S, Barone I, Giordano C, Bonofiglio D, Catalano S. The Multifaceted Mechanism of Leptin Signaling within Tumor Microenvironment in Driving Breast Cancer Growth and Progression. *Front Oncol*. 2014;4:340-48.
- 20: Simpson ER, Brown KA. Obesity and breast cancer: role of inflammation and aromatase. *J Mol Endocrinol*. 2013;51(3):T51-9.
- 21: Djiogue S, Nwabo Kamdje AH, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, Seke Etet PF. Insulin resistance and cancer: the role of insulin and IGFs. *Endocr Relat Cancer*. 2013;20(1):R1-R17.
- 22: Mahmood K, Naeem M, Rahimnajjad NA. Metformin: the hidden chronicles of a magic drug. *Eur J Intern Med*. 2013;24(1):20-6.
- 23: Gueron G, De Siervi A, Vazquez E. Advanced prostate cancer: reinforcing the strings between inflammation and the metastatic behavior. *Prostate Cancer Prostatic Dis*. 2012;15(3):213-21.
- 24: Contreras J, Rao DS. MicroRNAs in inflammation and immune responses. *Leukemia*. 2012;26(3):404-13.
- 25: Wu Y, Liu F. Targeting mTOR: evaluating the therapeutic potential of resveratrol for cancer treatment. *Anticancer Agents Med Chem*. 2013;13(7):1032-8.
- 26: Wojcik S. Crosstalk between autophagy and proteasome protein degradation systems: possible implications for cancer therapy. *Folia Histochem Cytobiol*. 2013;51(4):249-64.
- 27: Pal S, Bhattacharjee A, Ali A, Mandal NC, Mandal SC, Pal M. Chronic inflammation and cancer: potential chemoprevention through nuclear factor kappa B and p53 mutual antagonism. *J Inflamm (Lond)*. 2014;11:23-31.
- 28: Blaylock RL. Cancer microenvironment, inflammation and cancer stem cells: A hypothesis for a paradigm change and new targets in cancer control. *Surg Neurol Int*. 2015;6:92-98.

- 29: Hocking S, Samocha-Bonet D, Milner KL, Greenfield JR, Chisholm DJ. Adiposity and insulin resistance in humans: the role of the different tissue and cellular lipid depots. *Endocr Rev.* 2013;34(4):463-500.
- 30: Molica F, Morel S, Kwak BR, Rohner-Jeanrenaud F, Steffens S. Adipokines at the crossroad between obesity and cardiovascular disease. *Thromb Haemost.* 2015;113(3):553-66.
- 31: Marcadenti A, de Abreu-Silva EO. Different adipose tissue depots: Metabolic implications and effects of surgical removal. *Endocrinol Nutr.* 2015;62(9):458-64.
- 32: Lastra G, Manrique C. Perivascular adipose tissue, inflammation and insulin resistance: link to vascular dysfunction and cardiovascular disease. *Horm Mol Biol Clin Investig.* 2015;22(1):19-26.
- 33: Podolska MJ, Biermann MH, Maueröder C, Hahn J, Herrmann M. Inflammatory etiopathogenesis of systemic lupus erythematosus: an update. *J Inflamm Res.* 2015;8:161-71.
- 34: Toussirost É, Michel F, Binda D, Dumoulin G. The role of leptin in the pathophysiology of rheumatoid arthritis. *Life Sci.* 2015;140:29-36.
- 35: Verdile G, Keane KN, Cruzat VF, Medic S, Sabale M, Rowles J, Wijesekara N, Martins RN, Fraser PE, Newsholme P. Inflammation and Oxidative Stress: The Molecular Connectivity between Insulin Resistance, Obesity, and Alzheimer's Disease. *Mediators Inflamm.* 2015;2015:105828-42.
- 36: Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci.* 2014;69 Suppl 1:S4-9.
- 37: Yoo HJ, Choi KM. Adipokines as a novel link between obesity and atherosclerosis. *World J Diabetes.* 2014;5(3):357-63.
- 38: Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos. *Ann N Y Acad Sci.* 2012;1254:18-32.
- 39: Winnik S, Stein S, Matter CM. SIRT1 - an anti-inflammatory pathway at the crossroads between metabolic disease and atherosclerosis. *Curr Vasc Pharmacol.* 2012;10(6):693-6.
- 40: Shao BZ, Han BZ, Zeng YX, Su DF, Liu C. The roles of macrophage autophagy in atherosclerosis. *Acta Pharmacol Sin.* 2016. doi: 10.1038/aps.2015.87.
- 41: Sesti F, Tsitsilonis OE, Kotsinas A, Trougakos IP. Oxidative stress-mediated biomolecular damage and inflammation in tumorigenesis. *In Vivo.* 2012;26(3):395-402.
- 42: Erol A. Systemic DNA damage response and metabolic syndrome as a premalignant state. *Curr Mol Med.* 2010;10(3):321-34.
- 43: Schultz MA, Abdel-Mageed AB, Mondal D. The nrf1 and nrf2 balance in oxidative stress regulation and androgen signaling in prostate cancer cells. *Cancers(Basel).* 2010;2(2):1354-78.

- 44: Muinelo-Romay L, Alonso-Alconada L, Alonso-Nocelo M, Barbazan J, Abal M. Tumor invasion and oxidative stress: biomarkers and therapeutic strategies. *Curr Mol Med*. 2012;12(6):746-62.
- 45: Severi T, van Malenstein H, Verslype C, van Pelt JF. Tumor initiation and progression in hepatocellular carcinoma: risk factors, classification, and therapeutic targets. *Acta Pharmacol Sin*. 2010;31(11):1409-20.
- 46: Winner D, Norton L, Kanat M, Arya R, Fourcaudot M, Hansis-Diarte A, Tripathy D, DeFronzo RA, Jenkinson CP, Abdul-Ghani M. Strong association between insulin-mediated glucose uptake and the 2-hour, not the fasting plasma glucose concentration, in the normal glucose tolerance range. *J Clin Endocrinol Metab*. 2014;99(9):3444-9.
- 47: van Genugten RE, Serné EH, Heymans MW, van Raalte DH, Diamant M. Postprandial microvascular function deteriorates in parallel with gradual worsening of insulin sensitivity and glucose tolerance in men with the metabolic syndrome or type 2 diabetes. *Diabetologia*. 2013;56(3):583-7.
- 48: Lantier L, Williams AS, Williams IM, Yang KK, Bracy DP, Goelzer M, James FD, Gius D, Wasserman DH. SIRT3 Is Crucial for Maintaining Skeletal Muscle Insulin Action and Protects Against Severe Insulin Resistance in High-Fat-Fed Mice. *Diabetes*. 2015;64(9):3081-92.
- 49: Liu J, Zhang W, Chuang GC, Hill HS, Tian L, Fu Y, Moellering DR, Garvey WT. Role of TRIB3 in regulation of insulin sensitivity and nutrient metabolism during short-term fasting and nutrient excess. *Am J Physiol Endocrinol Metab*. 2012;303(7):E908-16.
- 50: Palsson-McDermott EM, O'Neill LA. The Warburg effect then and now: from cancer to inflammatory diseases. *Bioessays*. 2013;35(11):965-73.
- 51: Zhang C, Cao S, Toole BP, Xu Y. Cancer may be a pathway to cell survival under persistent hypoxia and elevated ROS: a model for solid-cancer initiation and early development. *Int J Cancer*. 2015;136(9):2001-11.
- 52: Schwarzer R, Dames S, Tondera D, Klippel A, Kaufmann J. TRB3 is a PI 3-kinase dependent indicator for nutrient starvation. *Cell Signal*. 2006;18(6):899-909.
- 53: Courtney R, Ngo DC, Malik N, Ververis K, Tortorella SM, Karagiannis TC. Cancer metabolism and the Warburg effect: the role of HIF-1 and PI3K. *Mol Biol Rep*. 2015;42(4):841-51.
- 54: Woo YM, Shin Y, Lee EJ, Lee S, Jeong SH, Kong HK, Park EY, Kim HK, Han J, Chang M, Park JH. Inhibition of Aerobic Glycolysis Represses Akt/mTOR/HIF-1 α Axis and Restores Tamoxifen Sensitivity in Antiestrogen-Resistant Breast Cancer Cells. *PLoS One*. 2015;10(7):e0132285.
- 55: Zhang J, Wen HJ, Guo ZM, Zeng MS, Li MZ, Jiang YE, He XG, Sun CZ. TRB3 overexpression due to endoplasmic reticulum stress inhibits AKT kinase activation of tongue squamous cell carcinoma. *Oral Oncol*. 2011;47(10):934-9.

- 56: Erazo T, Lorente M, López-Plana A, Muñoz-Guardiola P, Fernandez-Nogueira P, García-Martínez JA, Bragado P, Fuster G, Salazar M, Espadaler J, Hernandez-Losa J, Bayascas JR, Cortal M, Vidal L, Gascon P, Gomez-Ferreria M, Alfón J, Velasco G, Domenech C, Lizcano JM. The new antitumor drug ABTL0812 inhibits the Akt/mTORC1 axis by upregulating Tribbles-3 pseudokinase. *Clin Cancer Res.* 2015. pii: clincanres.1808.2015.
- 57: Rzymiski T, Paantjens A, Bod J, Harris AL. Multiple pathways are involved in the anoxia response of SKIP3 including HuR-regulated RNA stability, NF-kappaB and ATF4. *Oncogene.* 2008;27(33):4532-43.
- 58: Wennemers M, Bussink J, Scheijen B, Nagtegaal ID, van Laarhoven HW, Raleigh JA, Varia MA, Heuvel JJ, Rouschop KM, Sweep FC, Span PN. Tribbles homolog 3 denotes a poor prognosis in breast cancer and is involved in hypoxia response. *Breast Cancer Res.* 2011;13(4):R82. doi: 10.1186/bcr2934.
- 59: Dandekar A, Mendez R, Zhang K. Cross talk between ER stress, oxidative stress, and inflammation in health and disease. *Methods Mol Biol.* 2015;1292:205-14.
- 60: Jia G, Aroor AR, Martinez-Lemus LA, Sowers JR. Overnutrition, mTOR signaling, and cardiovascular diseases. *Am J Physiol Regul Integr Comp Physiol.* 2014;307(10):R1198-206.
- 61: Karsli-Uzunbas G, Guo JY, Price S, Teng X, Laddha SV, Khor S, Kalaany NY, Jacks T, Chan CS, Rabinowitz JD, White E. Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discov.* 2014;4(8):914-27.
- 62: White E, Mehnert JM, Chan CS. Autophagy, Metabolism, and Cancer. *Clin Cancer Res.* 2015;21(22):5037-46.
- 63: Chevet E, Hetz C, Samali A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. *Cancer Discov.* 2015;5(6):586-97.
- 64: Fang N, Zhang W, Xu S, Lin H, Wang Z, Liu H, Fang Q, Li C, Peng L, Lou J. TRIB3 alters endoplasmic reticulum stress-induced β -cell apoptosis via the NF- κ B pathway. *Metabolism.* 2014;63(6):822-30.
- 65: de Lorenzo C, Greco A, Fiorentino TV, Mannino GC, Hribal ML. Variants of insulin-signaling inhibitor genes in type 2 diabetes and related metabolic abnormalities. *Int J Genomics.* 2013;2013:376454. doi: 10.1155/2013/376454.
- 66: Hua F, Hu ZW. TRIB3-P62 interaction, diabetes and autophagy. *Oncotarget.* 2015;6(33):34061-2.
- 67: Salazar M, Lorente M, García-Taboada E, Pérez Gómez E, Dávila D, Zúñiga-García P, María Flores J, Rodríguez A, Hegedus Z, Mosén-Ansorena D, Aransay AM, Hernández-Tiedra S, López-Valero I, Quintanilla M, Sánchez C, Iovanna JL, Dusetti N, Guzmán M, Francis SE, Carracedo A, Kiss-Toth E, Velasco G. Loss of Tribbles pseudokinase-3 promotes Akt-driven tumorigenesis via FOXO inactivation. *Cell Death Differ.* 2015;22(1):131-44.

- 68: 1: Smith SM, Moran AP, Duggan SP, Ahmed SE, Mohamed AS, Windle HJ, O'Neill LA, Kelleher DP. Tribbles 3: a novel regulator of TLR2-mediated signaling in response to *Helicobacter pylori* lipopolysaccharide. *J Immunol.* 2011;186(4):2462-71.
- 69: Mata J, Curado S, Ephrussi A, Rørth P. Tribbles coordinates mitosis and morphogenesis in *Drosophila* by regulating string/CDC25 proteolysis. *Cell.* 2000;101(5):511-22.
- 70: Seher TC, Leptin M. Tribbles, a cell-cycle brake that coordinates proliferation and morphogenesis during *Drosophila* gastrulation. *Curr Biol.* 2000;10(11):623-9.
- 71: Grosshans J, Wieschaus E. A genetic link between morphogenesis and cell division during formation of the ventral furrow in *Drosophila*. *Cell.* 2000;101(5):523-31.
- 72: Saka Y, Smith JC. A *Xenopus* tribbles orthologue is required for the progression of mitosis and for development of the nervous system. *Dev Biol.* 2004;273(2):210-25.
- 73: Farrell JA, O'Farrell PH. Mechanism and regulation of Cdc25/Twine protein destruction in embryonic cell-cycle remodeling. *Curr Biol.* 2013;23(2):118-26
- 74: Masoner V, Das R, Pence L, Anand G, LaFerriere H, Zars T, Bouyain S, Dobens LL. The kinase domain of *Drosophila* Tribbles is required for turnover of fly C/EBP during cell migration. *Dev Biol.* 2013;375(1):33-44.
- 75: Rørth P, Szabo K, Texido G. The level of C/EBP protein is critical for cell migration during *Drosophila* oogenesis and is tightly controlled by regulated degradation. *Mol Cell.* 2000;6(1):23-30.
- 76: Manea SA, Todirita A, Manea A. High glucose-induced increased expression of endothelin-1 in human endothelial cells is mediated by activated CCAAT/enhancer-binding proteins. *PLoS One.* 2013;8(12):e84170.
- 77: Jiang C, Sun J, Dai Y, Cao P, Zhang L, Peng S, Zhou Y, Li G, Tang J, Xiang J. HIF-1A and C/EBPs transcriptionally regulate adipogenic differentiation of bone marrow-derived MSCs in hypoxia. *Stem Cell Res Ther.* 2015;6:21. doi:10.1186/s13287-015-0014-4.
- 78: Manea SA, Todirita A, Raicu M, Manea A. C/EBP transcription factors regulate NADPH oxidase in human aortic smooth muscle cells. *J Cell Mol Med.* 2014;18(7):1467-77.
- 79: Dolfini D, Mantovani R. Targeting the Y/CCAAT box in cancer: YB-1 (YBX1) or NF-Y? *Cell Death Differ.* 2013;20(5):676-85.
- 80: Salomè M, Campos J, Keeshan K. TRIB2 and the ubiquitin proteasome system in cancer. *Biochem Soc Trans.* 2015;43(5):1089-94.
- 81: Bowers AJ, Scully S, Boylan JF. SKIP3, a novel *Drosophila* tribbles ortholog, is overexpressed in human tumors and is regulated by hypoxia. *Oncogene.* 2003;22(18):2823-35.

- 82: Kiss-Toth E, Bagstaff SM, Sung HY, Jozsa V, Dempsey C, Caunt JC, Oxley KM, Wyllie DH, Polgar T, Harte M, O'Neill LA, Qwarnstrom EE, Dower SK. Human tribbles, a protein family controlling mitogen-activated protein kinase cascades. *J Biol Chem*. 2004;279(41):42703-8.
- 83: Cunard R. Mammalian tribbles homologs at the crossroads of endoplasmic reticulum stress and Mammalian target of rapamycin pathways. *Scientifica (Cairo)*. 2013;2013:750871.
- 84: Hegedus Z, Czibula A, Kiss-Toth E. Tribbles: a family of kinase-like proteins with potent signaling regulatory function. *Cell Signal*. 2007;19(2):238-50.
- 85: Sung HY, Guan H, Czibula A, King AR, Eder K, Heath E, Suvarna SK, Dower SK, Wilson AG, Francis SE, Crossman DC, Kiss-Toth E. Human tribbles-1 controls proliferation and chemotaxis of smooth muscle cells via MAPK signaling pathways. *J Biol Chem*. 2007;282(25):18379-87.
- 86: Link W. Tribbles breaking bad: TRIB2 suppresses FOXO and acts as an oncogenic protein in melanoma. *Biochem Soc Trans*. 2015;43(5):1085-8.
- 87: Xu S, Tong M, Huang J, Zhang Y, Qiao Y, Weng W, Liu W, Wang J, Sun F. TRIB2 inhibits Wnt/ β -Catenin/TCF4 signaling through its associated ubiquitin E3 ligases, β -TrCP, COP1 and Smurf1, in liver cancer cells. *FEBS Lett*. 2014;588(23):4334-41.
- 88: Qian B, Wang H, Men X, Zhang W, Cai H, Xu S, Xu Y, Ye L, Wollheim CB, Lou J. TRIB3 is implicated in glucotoxicity- and endoplasmic reticulum-stress-induced beta-cell apoptosis. *J Endocrinol*. 2008;199(3):407-16.
- 89: Wennemers M, Bussink J, Grebenchtchikov N, Sweep FC, Span PN. TRIB3 protein denotes a good prognosis in breast cancer patients and is associated with hypoxia sensitivity. *Radiother Oncol*. 2011;101(1):198-202.
- 90: Prudente S, Morini E, Trischitta V. The emerging role of TRIB3 as a gene affecting human insulin resistance and related clinical outcomes. *Acta Diabetol*. 2009;46(2):79-84.
- 91: Prudente S, Sesti G, Pandolfi A, Andreozzi F, Consoli A, Trischitta V. The mammalian tribbles homolog TRIB3, glucose homeostasis, and cardiovascular diseases. *Endocr Rev*. 2012;33(4):526-46.
- 92: Corcoran CA, Luo X, He Q, Jiang C, Huang Y, Sheikh MS. Genotoxic and endoplasmic reticulum stresses differentially regulate TRB3 expression. *Cancer Biol Ther*. 2005;4(10):1063-7.
- 93: Chhabra R, Dubey R, Saini N. Gene expression profiling indicate role of ER stress in miR-23a~27a~24-2 cluster induced apoptosis in HEK293T cells. *RNA Biol*. 2011;8(4):648-64.
- 94: Shang YY, Zhong M, Zhang LP, Guo ZX, Wang ZH, Zhang Y, Deng JT, Zhang W. Tribble 3, a novel oxidized low-density lipoprotein-inducible gene, is induced via the activating transcription factor 4-C/EBP homologous protein pathway. *Clin Exp Pharmacol Physiol*. 2010;37(1):51-5.

- 95: Nicoletti-Carvalho JE, Nogueira TC, Gorjão R, Bromati CR, Yamanaka TS, Boschero AC, Velloso LA, Curi R, Anhê GF, Bordin S. UPR-mediated TRIB3 expression correlates with reduced AKT phosphorylation and inability of interleukin 6 to overcome palmitate-induced apoptosis in RINm5F cells. *J Endocrinol.* 2010;206(2):183-93.
- 96: Wennemers M, Bussink J, van den Beucken T, Sweep FC, Span PN. Regulation of TRIB3 mRNA and protein in breast cancer. *PLoS One.* 2012;7(11):e49439.
- 97: Örd T, Innos J, Lilleväli K, Tekko T, Sütt S, Örd D, Kõks S, Vasar E, Örd T. Trib3 is developmentally and nutritionally regulated in the brain but is dispensable for spatial memory, fear conditioning and sensing of amino acid-imbalanced diet. *PLoS One.* 2014;9(4):e94691.
- 98: Kim S, Hata A, Kang H. Down-regulation of miR-96 by bone morphogenetic protein signaling is critical for vascular smooth muscle cell phenotype modulation. *J Cell Biochem.* 2014;115(5):889-95.
- 99: Hua F, Mu R, Liu J, Xue J, Wang Z, Lin H, Yang H, Chen X, Hu Z. TRB3 interacts with SMAD3 promoting tumor cell migration and invasion. *J Cell Sci.* 2011;124(Pt 19):3235-46.
- 100: Douvris A, Soubeyrand S, Naing T, Martinuk A, Nikpay M, Williams A, Buick J, Yauk C, McPherson R. Functional analysis of the TRIB1 associated locus linked to plasma triglycerides and coronary artery disease. *J Am Heart Assoc.* 2014;3(3):e000884.
- 101: Imajo M, Nishida E. Human Tribbles homolog 1 functions as a negative regulator of retinoic acid receptor. *Genes Cells.* 2010;15(10):1089-97.
- 102: Satoh T, Kidoya H, Naito H, Yamamoto M, Takemura N, Nakagawa K, Yoshioka Y, Morii E, Takakura N, Takeuchi O, Akira S. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. *Nature.* 2013;495(7442):524-8.
- 103: Katzav A, Arango MT, Kivity S, Tanaka S, Givaty G, Agmon-Levin N, Honda M, Anaya JM, Chapman J, Shoenfeld Y. Passive transfer of narcolepsy: anti-TRIB2 autoantibody positive patient IgG causes hypothalamic orexin neuron loss and sleep attacks in mice. *J Autoimmun.* 2013;45:24-30.
- 104: Keeshan K, He Y, Wouters BJ, Shestova O, Xu L, Sai H, Rodriguez CG, Maillard I, Tobias JW, Valk P, Carroll M, Aster JC, Delwel R, Pear WS. Tribbles homolog 2 inactivates C/EBPalpha and causes acute myelogenous leukemia. *Cancer Cell.* 2006;10(5):401-11.
- 105: Xu S, Tong M, Huang J, Zhang Y, Qiao Y, Weng W, Liu W, Wang J, Sun F. TRIB2 inhibits Wnt/ β -Catenin/TCF4 signaling through its associated ubiquitin E3 ligases, β -TrCP, COP1 and Smurf1, in liver cancer cells. *FEBS Lett.* 2014;588(23):4334-41.
- 106: Link W. Tribbles breaking bad: TRIB2 suppresses FOXO and acts as an oncogenic protein in melanoma. *Biochem Soc Trans.* 2015;43(5):1085-8.

- 107: Izrailit J, Berman HK, Datti A, Wrana JL, Reedijk M. High throughput kinase inhibitor screens reveal TRB3 and MAPK-ERK/TGF β pathways as fundamental Notch regulators in breast cancer. *Proc Natl Acad Sci U S A*. 2013;110(5):1714-9.
- 108: Salazar M, Lorente M, García-Taboada E, Hernández-Tiedra S, Davila D, Francis SE, Guzmán M, Kiss-Toth E, Velasco G. The pseudokinase tribbles homologue-3 plays a crucial role in cannabinoid anticancer action. *Biochim Biophys Acta*. 2013;1831(10):1573-8.
- 109: Li T, Su L, Zhong N, Hao X, Zhong D, Singhal S, Liu X. Salinomycin induces cell death with autophagy through activation of endoplasmic reticulum stress in human cancer cells. *Autophagy*. 2013;9(7):1057-68.
- 110: Mathur A, Abd Elmageed ZY, Liu X, Kostochka ML, Zhang H, Abdel-Mageed AB, Mondal D. Subverting ER-stress towards apoptosis by nelfinavir and curcumin coexposure augments docetaxel efficacy in castration resistant prostate cancer cells. *PLoS One*. 2014;9(8):e103109.
- 111: Ohoka N, Hattori T, Kitagawa M, Onozaki K, Hayashi H. Critical and functional regulation of CHOP (C/EBP homologous protein) through the N-terminal portion. *J Biol Chem*. 2007;282(49):35687-94.
- 112: Brisard D, Chesnel F, Elis S, Desmarchais A, Sánchez-Lazo L, Chasles M, Maillard V, Uzbekova S. Tribbles expression in cumulus cells is related to oocyte maturation and fatty acid metabolism. *J Ovarian Res*. 2014;7:44-56.
- 113: Zou T, Liu WJ, Li SD, Zhou W, Yang JF, Zou CG. TRB3 mediates homocysteine-induced inhibition of endothelial cell proliferation. *J Cell Physiol*. 2011;226(11):2782-9.
- 114: Guo L, Guo ZX, Gong HP, Shang YY, Zhong M, Zhang Y, Zhang W. Tribbles homolog 3 is induced by high glucose and associated with apoptosis in human endothelial cells. *Mol Med Rep*. 2015;12(2):1963-70.
- 115: Wang ZH, Shang YY, Zhang S, Zhong M, Wang XP, Deng JT, Pan J, Zhang Y, Zhang W. Silence of TRIB3 suppresses atherosclerosis and stabilizes plaques in diabetic ApoE $^{-/-}$ /LDL receptor $^{-/-}$ mice. *Diabetes*. 2012;61(2):463-73.
- 116: Bi XP, Tan HW, Xing SS, Wang ZH, Tang MX, Zhang Y, Zhang W. Overexpression of TRB3 gene in adipose tissue of rats with high fructose-induced metabolic syndrome. *Endocr J*. 2008;55(4):747-52.
- 117: Ord T, Ord D, Kuuse S, Plaas M, Ord T. Trib3 is regulated by IL-3 and affects bone marrow-derived mast cell survival and function. *Cell Immunol*. 2012;280(1):68-75.
- 118: Shang YY, Wang ZH, Zhang LP, Zhong M, Zhang Y, Deng JT, Zhang W. TRB3, upregulated by ox-LDL, mediates human monocyte-derived macrophage apoptosis. *FEBS J*. 2009;276(10):2752-61.

- 119: Berisha SZ, Hsu J, Robinet P, Smith JD. Transcriptome analysis of genes regulated by cholesterol loading in two strains of mouse macrophages associates lysosome pathway and ER stress response with atherosclerosis susceptibility. *PLoS One*. 2013;8(5):e65003.
- 120: Beguinot F. Tribbles homologue 3 (TRIB3) and the insulin-resistance genes in type 2 diabetes. *Diabetologia*. 2010;53(9):1831-4.
- 121: Örd T, Örd D, Adler P, Vilo J, Örd T. TRIB3 enhances cell viability during glucose deprivation in HEK293-derived cells by upregulating IGFBP2, a novel nutrient deficiency survival factor. *Biochim Biophys Acta*. 2015;1853(10 PtA):2492-505.
- 122: Lima AF, Ropelle ER, Pauli JR, Cintra DE, Frederico MJ, Pinho RA, Velloso LA, De Souza CT. Acute exercise reduces insulin resistance-induced TRB3 expression and amelioration of the hepatic production of glucose in the liver of diabetic mice. *J Cell Physiol*. 2009;221(1):92-7.
- 123: Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science*. 2003;300(5625):1574-7.
- 124: Sakai S, Ohoka N, Onozaki K, Kitagawa M, Nakanishi M, Hayashi H. Dual mode of regulation of cell division cycle 25 A protein by TRB3. *Biol Pharm Bull*. 2010;33(7):1112-6.
- 125: Zhou Y, Li L, Liu Q, Xing G, Kuai X, Sun J, Yin X, Wang J, Zhang L, He F. E3 ubiquitin ligase SIAH1 mediates ubiquitination and degradation of TRB3. *Cell Signal*. 2008;20(5):942-8.
- 126: Ord D, Meerits K, Ord T. TRB3 protects cells against the growth inhibitory and cytotoxic effect of ATF4. *Exp Cell Res*. 2007;313(16):3556-67.
- 127: Kristiansen M, Menghi F, Hughes R, Hubank M, Ham J. Global analysis of gene expression in NGF-deprived sympathetic neurons identifies molecular pathways associated with cell death. *BMC Genomics*. 2011;12:551-62.
- 128: Zareen N, Biswas SC, Greene LA. A feed-forward loop involving Trib3, Akt and FoxO mediates death of NGF-deprived neurons. *Cell Death Differ*. 2013;20(12):1719-30.
- 129: Ono M, Ohkouchi S, Kanehira M, Tode N, Kobayashi M, Ebina M, Nukiwa T, Irokawa T, Ogawa H, Akaike T, Okada Y, Kurosawa H, Kikuchi T, Ichinose M. Mesenchymal stem cells correct inappropriate epithelial-mesenchyme relation in pulmonary fibrosis using stanniocalcin-1. *Mol Ther*. 2015;23(3):549-60.
- 130: Zhang Y, Luo F, Wu S, Yu B, Liu T, Wu Y. Tribbles homolog 3 expression in spermatogonial stem cells of rat testes. *Cell Biol Int*. 2014;38(12):1403-7.
- 131: Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q, Gupta PB. Epithelial-to-mesenchymal transition activates PERK-eIF2 α and sensitizes cells to endoplasmic reticulum stress. *Cancer Discov*. 2014;4(6):702-15.

- 132: Wielenga MC, Colak S, Heijmans J, van Lidth de Jeude JF, Rodermond HM, Paton JC, Paton AW, Vermeulen L, Medema JP, van den Brink GR. ER-Stress-Induced Differentiation Sensitizes Colon Cancer Stem Cells to Chemotherapy. *Cell Rep.* 2015;13(3):490-4.
- 133: Niederreiter L, Fritz TM, Adolph TE, Krismer AM, Offner FA, Tschurtschenthaler M, Flak MB, Hosomi S, Tomczak MF, Kaneider NC, Sarcevic E, Kempster SL, Raine T, Esser D, Rosenstiel P, Kohno K, Iwawaki T, Tilg H, Blumberg RS, Kaser A. ER stress transcription factor Xbp1 suppresses intestinal tumorigenesis and directs intestinal stem cells. *J Exp Med.* 2013;210(10):2041-56.
- 134: Miyoshi N, Ishii H, Mimori K, Takatsuno Y, Kim H, Hirose H, Sekimoto M, Doki Y, Mori M. Abnormal expression of TRIB3 in colorectal cancer: a novel marker for prognosis. *Br J Cancer.* 2009;101(10):1664-70.
- 135: Zhou H, Luo Y, Chen JH, Hu J, Luo YZ, Wang W, Zeng Y, Xiao L. Knockdown of TRB3 induces apoptosis in human lung adenocarcinoma cells through regulation of Notch 1 expression. *Mol Med Rep.* 2013;8(1):47-52.
- 136: Locatelli SL, Giacomini A, Guidetti A, Cleris L, Mortarini R, Anichini A, Gianni AM, Carlo-Stella C. Perifosine and sorafenib combination induces mitochondrial cell death and antitumor effects in NOD/SCID mice with Hodgkin lymphoma cell line xenografts. *Leukemia.* 2013;27(8):1677-87.
- 137: Salazar M, Carracedo A, Salanueva IJ, Hernández-Tiedra S, Lorente M, Egia A, Vázquez P, Blázquez C, Torres S, García S, Nowak J, Fimia GM, Piacentini M, Cecconi F, Pandolfi PP, González-Feria L, Iovanna JL, Guzmán M, Boya P, Velasco G. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *J Clin Invest.* 2009;119(5):1359-72.
- 138: Wu M, Xu LG, Zhai Z, Shu HB. SINK is a p65-interacting negative regulator of NF-kappaB-dependent transcription. *J Biol Chem.* 2003;278(29):27072-9.
- 139: Huang J, Teng L, Liu T, Li L, Chen D, Li F, Xu LG, Zhai Z, Shu HB. Identification of a novel serine/threonine kinase that inhibits TNF-induced NF-kappaB activation and p53-induced transcription. *Biochem Biophys Res Commun.* 2003;309(4):774-8.
- 140: Kiss-Toth E, Bagstaff SM, Sung HY, Jozsa V, Dempsey C, Caunt JC, Oxley KM, Wyllie DH, Polgar T, Harte M, O'Neill LA, Qwarnstrom EE, Dower SK. Human tribbles, a protein family controlling mitogen-activated protein kinase cascades. *J Biol Chem.* 2004;279(41):42703-8.
- 141: Ghatak S, Morgner J, Wickström SA. ILK: a pseudokinase with a unique function in the integrin-actin linkage. *Biochem Soc Trans.* 2013;41(4):995-1001.
- 142: Silvennoinen O, Ungureanu D, Niranjana Y, Hammaren H, Bandaranayake R, Hubbard SR. New insights into the structure and function of the pseudokinase domain in JAK2. *Biochem Soc Trans.* 2013;41(4):1002-7.

- 143: Takahashi Y, Ohoka N, Hayashi H, Sato R. TRB3 suppresses adipocyte differentiation by negatively regulating PPARgamma transcriptional activity. *J Lipid Res.* 2008;49(4):880-92.
- 144: Ohoka N, Sakai S, Onozaki K, Nakanishi M, Hayashi H. Anaphase-promoting complex/cyclosome-cdh1 mediates the ubiquitination and degradation of TRB3. *Biochem Biophys Res Commun.* 2010;392(3):289-94.
- 145: Weismann D, Erion DM, Ignatova-Todorava I, Nagai Y, Stark R, Hsiao JJ, Flannery C, Birkenfeld AL, May T, Kahn M, Zhang D, Yu XX, Murray SF, Bhanot S, Monia BP, Cline GW, Shulman GI, Samuel VT. Knockdown of the gene encoding *Drosophila* tribbles homologue 3 (Trib3) improves insulin sensitivity through peroxisome proliferator-activated receptor- γ (PPAR- γ) activation in a rat model of insulin resistance. *Diabetologia.* 2011;54(4):935-44.
- 146: Dedhia PH, Keeshan K, Uljon S, Xu L, Vega ME, Shestova O, Zaks-Zilberman M, Romany C, Blacklow SC, Pear WS. Differential ability of Tribbles family members to promote degradation of C/EBPalpha and induce acute myelogenous leukemia. *Blood.* 2010;116(8):1321-8.
- 147: Lampiasi N, Azzolina A, Umezawa K, Montalto G, McCubrey JA, Cervello M. The novel NF- κ B inhibitor DHMEQ synergizes with celecoxib to exert antitumor effects on human liver cancer cells by a ROS-dependent mechanism. *Cancer Lett.* 2012;322(1):35-44.
- 148: Hua F, Li K, Yu JJ, Hu ZW. The TRIB3-SQSTM1 interaction mediates metabolic stress-promoted tumorigenesis and progression via suppressing autophagic and proteasomal degradation. *Autophagy.* 2015;11(10):1929-31.
- 149: Aynaud MM, Suspène R, Vidalain PO, Mussil B, Guétard D, Tangy F, Wain-Hobson S, Vartanian JP. Human Tribbles 3 protects nuclear DNA from cytidine deamination by APOBEC3A. *J Biol Chem.* 2012;287(46):39182-92.
- 150: Wang J, Wu Z, Li D, Li N, Dindot SV, Satterfield MC, Bazer FW, Wu G. Nutrition, epigenetics, and metabolic syndrome. *Antioxid Redox Signal.* 2012;17(2):282-301.
- 151: Zhang H, Dellsperger KC, Zhang C. The link between metabolic abnormalities and endothelial dysfunction in type 2 diabetes: an update. *Basic Res Cardiol.* 2012;107(1):237-43.
- 152: Kim OK, Jun W, Lee J. Mechanism of ER Stress and Inflammation for Hepatic Insulin Resistance in Obesity. *Ann Nutr Metab.* 2015;67(4):218-27.
- 153: Nolan CJ, Ruderman NB, Kahn SE, Pedersen O, Prentki M. Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. *Diabetes.* 2015;64(3):673-86.
- 154: Guerrero-Hernández A, Leon-Aparicio D, Chavez-Reyes J, Olivares-Reyes JA, DeJesus S. Endoplasmic reticulum stress in insulin resistance and diabetes. *Cell Calcium.* 2014;56(5):311-22.

- 155: Dicks N, Gutierrez K, Michalak M, Bordignon V, Agellon LB. Endoplasmic reticulum stress, genome damage, and cancer. *Front Oncol.* 2015;5:11-22.
- 156: Maurel M, McGrath EP, Mnich K, Healy S, Chevet E, Samali A. Controlling the unfolded protein response-mediated life and death decisions in cancer. *Semin Cancer Biol.* 2015;33:57-66.
- 157: Dejeans N, Barroso K, Fernandez-Zapico ME, Samali A, Chevet E. Novel roles of the unfolded protein response in the control of tumor development and aggressiveness. *Semin Cancer Biol.* 2015;33:67-73.
- 158: Zanetti M, Rodvold JJ, Mahadevan NR. The evolving paradigm of cell-nonautonomous UPR-based regulation of immunity by cancer cells. *Oncogene.* 2016;35(3):269-78.
- 159: Nagelkerke A, Bussink J, Sweep FC, Span PN. The unfolded protein response as a target for cancer therapy. *Biochim Biophys Acta.* 2014;1846(2):277-84.
- 160: Bromati CR, Lellis-Santos C, Yamanaka TS, Nogueira TC, Leonelli M, Caperuto LC, Gorjão R, Leite AR, Anhê GF, Bordin S. UPR induces transient burst of apoptosis in islets of early lactating rats through reduced AKT phosphorylation via ATF4/CHOP stimulation of TRB3 expression. *Am J Physiol Regul Integr Comp Physiol.* 2011;300(1):R92-100.
- 161: Salazar M, Carracedo A, Salanueva IJ, Hernández-Tiedra S, Egia A, Lorente M, Vázquez P, Torres S, Iovanna JL, Guzmán M, Boya P, Velasco G. TRB3 links ER stress to autophagy in cannabinoid anti-tumoral action. *Autophagy.* 2009;5(7):1048-9.
- 162: Ni M, Lee AS. ER chaperones in mammalian development and human diseases. *FEBS Lett.* 2007;581(19):3641-51.
- 163: Senkal CE, Ponnusamy S, Manevich Y, Meyers-Needham M, Saddoughi SA, Mukhopadhyay A, Dent P, Bielawski J, Ogretmen B. Alteration of ceramide synthase 6/C16-ceramide induces activating transcription factor 6-mediated endoplasmic reticulum (ER) stress and apoptosis via perturbation of cellular Ca²⁺ and ER/Golgi membrane network. *J Biol Chem.* 2011;286(49):42446-58.
- 164: Ochi T, Munekage K, Ono M, Higuchi T, Tsuda M, Hayashi Y, Okamoto N, Toda K, Sakamoto S, Oben JA, Saibara T. PNPLA3 is involved in hepatic fatty acid and triglyceride metabolism through XBP1 and modulation of endoplasmic reticulum stress in mice. *Hepatol Res.* 2015. doi: 10.1111/hepr.12587.
- 165: Duan Q, Chen C, Yang L, Li N, Gong W, Li S, Wang DW. MicroRNA regulation of unfolded protein response transcription factor XBP1 in the progression of cardiac hypertrophy and heart failure in vivo. *J Transl Med.* 2015;13(1):363-74.
- 166: Elouil H, Bensellam M, Guiot Y, Vander Mierde D, Pascal SM, Schuit FC, Jonas JC. Acute nutrient regulation of the unfolded protein response and integrated stress response in cultured rat pancreatic islets. *Diabetologia.* 2007;50(7):1442-52.

- 167: Guo FJ, Xiong Z, Lu X, Ye M, Han X, Jiang R. ATF6 upregulates XBP1S and inhibits ER stress-mediated apoptosis in osteoarthritis cartilage. *Cell Signal*. 2014;26(2):332-42.
- 168: Madhusudhan T, Wang H, Dong W, Ghosh S, Bock F, Thangapandi VR, Ranjan S, Wolter J, Kohli S, Shahzad K, Heidel F, Krueger M, Schwenger V, Moeller MJ, Kalinski T, Reiser J, Chavakis T, Isermann B. Defective podocyte insulin signalling through p85-XBP1 promotes ATF6-dependent maladaptive ER-stress response in diabetic nephropathy. *Nat Commun*. 2015;6:6496-7007.
- 170: Avivar-Valderas A, Bobrovnikova-Marjon E, Alan Diehl J, Bardeesy N, Debnath J, Aguirre-Ghiso JA. Regulation of autophagy during ECM detachment is linked to a selective inhibition of mTORC1 by PERK. *Oncogene*. 2013;32(41):4932-40.
- 171: Gao Y, Sartori DJ, Li C, Yu QC, Kushner JA, Simon MC, Diehl JA. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. *Mol Cell Biol*. 2012;32(24):5129-39.
- 172: Liu ZW, Zhu HT, Chen KL, Dong X, Wei J, Qiu C, Xue JH. Protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling pathway plays a major role in reactive oxygen species (ROS)-mediated endoplasmic reticulum stress-induced apoptosis in diabetic cardiomyopathy. *Cardiovasc Diabetol*. 2013;12:158-65.
- 173: Masuda M, Miyazaki-Anzai S, Levi M, Ting TC, Miyazaki M. PERK-eIF2 α -ATF4-CHOP signaling contributes to TNF α -induced vascular calcification. *J Am Heart Assoc*. 2013;2(5):e000238.
- 174: Kim EJ, Lee YJ, Kang S, Lim YB. Ionizing radiation activates PERK/eIF2 α /ATF4 signaling via ER stress-independent pathway in human vascular endothelial cells. *Int J Radiat Biol*. 2014;90(4):306-12.
- 175: Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H. TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. *EMBO J*. 2005;24(6):1243-55.
- 176: Ord D, Ord T. Characterization of human NIPK (TRB3, SKIP3) gene activation in stressful conditions. *Biochem Biophys Res Commun*. 2005;330(1):210-8.
- 177: Jousse C, Deval C, Maurin AC, Parry L, Chérasse Y, Chaveroux C, Lefloch R, Lenormand P, Bruhat A, Fafournoux P. TRB3 inhibits the transcriptional activation of stress-regulated genes by a negative feedback on the ATF4 pathway. *J Biol Chem*. 2007;282(21):15851-61.
- 178: Su N, Kilberg MS. C/EBP homology protein (CHOP) interacts with activating transcription factor 4 (ATF4) and negatively regulates the stress-dependent induction of the asparagine synthetase gene. *J Biol Chem*. 2008;283(50):35106-17.
- 179: Liew CW, Bochenski J, Kawamori D, Hu J, Leech CA, Wanic K, Malecki M, Warram JH, Qi L, Krolewski AS, Kulkarni RN. The pseudokinase tribbles homolog 3 interacts with ATF4 to negatively regulate insulin exocytosis in human and mouse beta cells. *J Clin Invest*. 2010;120(8):2876-88.

- 180: Palmer BF, Clegg DJ. Oxygen sensing and metabolic homeostasis. *Mol Cell Endocrinol.* 2014;397(1-2):51-8.
- 181: Liu H, Cao MM, Wang Y, Li LC, Zhu LB, Xie GY, Li YB. Endoplasmic reticulum stress is involved in the connection between inflammation and autophagy in type 2 diabetes. *Gen Comp Endocrinol.* 2015;210:124-9.
- 182: Ryter SW, Koo JK, Choi AM. Molecular regulation of autophagy and its implications for metabolic diseases. *Curr Opin Clin Nutr Metab Care.* 2014;17(4):329-37.
- 183: Xia P, Xu XY. PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application. *Am J Cancer Res.* 2015;5(5):1602-9.
- 184: Carneiro BA, Kaplan JB, Altman JK, Giles FJ, Plataniias LC. Targeting mTOR signaling pathways and related negative feedback loops for the treatment of acute myeloid leukemia. *Cancer Biol Ther.* 2015;16(5):648-56.
- 185: Zhang N, Cao MM, Liu H, Xie GY, Li YB. Autophagy regulates insulin resistance following endoplasmic reticulum stress in diabetes. *J Physiol Biochem.* 2015;71(2):319-27.
- 186: Okamoto H, Latres E, Liu R, Thabet K, Murphy A, Valenzeula D, Yancopoulos GD, Stitt TN, Glass DJ, Sleeman MW. Genetic deletion of Trb3, the mammalian *Drosophila* tribbles homolog, displays normal hepatic insulin signaling and glucose homeostasis. *Diabetes* 2007; 56(5): 1350-1356.
- 187: Liu J, Wu X, Franklin JL, Messina JL, Hills HS, Moellering DR, Walton RG, Martin M, Garvey WT. Mammalian Tribbles homolog 3 impairs insulin action in skeletal muscle : role in glucose-induced insulin resistance. *Am J Physiol Endocrinol Metab.* 2010; 298(3): E565-76
- 188: Mikawa T, LLeonart ME, Takaori-Kondo A, Inagaki N, Yokode M, Kondoh H. Dysregulated glycolysis as an oncogenic event. *Cell Mol Life Sci.* 2015;72(10):1881-92.
- 189: Bezy O, Vernochet C, Gesta S, Farmer SR, Kahn CR. TRB3 blocks adipocyte differentiation through the inhibition of C/EBPbeta transcriptional activity. *Mol Cell Biol.* 2007;27(19):6818-31.
- 190: Matsumoto M, Han S, Kitamura T, Accili D. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest.* 2006;116(9):2464-72.
- 191: Schneider CC, Ampofo E, Montenarh M. CK2 regulates ATF4 and CHOP transcription within the cellular stress response signalling pathway. *Cell Signal.* 2012;24(9):1797-802.
- 192: Carraro V, Maurin AC, Lambert-Langlais S, Averous J, Chaveroux C, Parry L, Jousse C, Ord D, Ord T, Fafournoux P, Bruhat A. Amino acid availability controls TRB3 transcription in liver through the GCN2/eIF2 α /ATF4 pathway. *PLoS One.* 2010;5(12):e15716.
- 193: Gills JJ, Lopiccolo J, Tsurutani J, Shoemaker RH, Best CJ, Abu-Asab MS, Borojerdi J, Warfel NA, Gardner ER, Danish M, Hollander MC, Kawabata S, Tsokos M, Figg WD, Steeg PS, Dennis PA.

Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis in vitro and in vivo. *Clin Cancer Res.* 2007;13(17):5183-94.

- 194: Johnson CE, Hunt DK, Wiltshire M, Herbert TP, Sampson JR, Errington RJ, Davies DM, Tee AR. Endoplasmic reticulum stress and cell death in mTORC1-overactive cells is induced by nelfinavir and enhanced by chloroquine. *Mol Oncol.* 2015;9(3):675-88.
- 195: Zhang C, Li B, Zhang X, Hazarika P, Aggarwal BB, Duvic M. Curcumin selectively induces apoptosis in cutaneous T-cell lymphoma cell lines and patients' PBMCs: potential role for STAT-3 and NF-kappaB signaling. *J Invest Dermatol.* 2010;130(8):2110-9.
- 196: Vallianou NG, Evangelopoulos A, Schizas N, Kazazis C. Potential anticancer properties and mechanisms of action of curcumin. *Anticancer Res.* 2015;35(2):645-51.
- 197: Matyszewski A, Czarnecka AM, Solarek W, Korzeń P, Safir IJ, Kukwa W, Szczalik C. Molecular basis of carcinogenesis in diabetic patients (review). *Int J Oncol.* 2015;46(4):1435-43.
- 198: Cheng Z, Tseng Y, White MF. Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol Metab.* 2010;21(10):589-98.
- 199: Isenović ER, Kedees MH, Tepavcević S, Milosavljević T, Korićanac G, Trpković A, Marche P. Role of PI3K/AKT, cPLA2 and ERK1/2 signaling pathways in insulin regulation of vascular smooth muscle cells proliferation. *Cardiovasc Hematol Disord Drug Targets.* 2009;9(3):172-80.
- 200: Krook A, Wallberg-Henriksson H, Zierath JR. Sending the signal: molecular mechanisms regulating glucose uptake. *Med Sci Sports Exerc.* 2004;36(7):1212-7.
- 201: Henriksen EJ. Dysregulation of glycogen synthase kinase-3 in skeletal muscle and the etiology of insulin resistance and type 2 diabetes. *Curr Diabetes Rev.* 2010;6(5):285-93.
- 202: Huang S, Zhu M, Wu W, Rashid A, Liang Y, Hou L, Ning Q, Luo X. Valproate pretreatment protects pancreatic β -cells from palmitate-induced ER stress and apoptosis by inhibiting glycogen synthase kinase-3 β . *J Biomed Sci.* 2014;21:38-45.
- 203: Feng L, Zhang D, Fan C, Ma C, Yang W, Meng Y, Wu W, Guan S, Jiang B, Yang M, Liu X, Guo D. ER stress-mediated apoptosis induced by celastrol in cancer cells and important role of glycogen synthase kinase-3 β in the signal network. *Cell Death Dis.* 2013;4:e715.
- 204: Liu WJ, Ma LQ, Liu WH, Zhou W, Zhang KQ, Zou CG. Inhibition of hepatic glycogen synthesis by hyperhomocysteinemia mediated by TRB3. *Am J Pathol.* 2011;178(4):1489-99.
- 205: Andreozzi F, Formoso G, Prudente S, Hribal ML, Pandolfi A, Bellacchio E, Di Silvestre S, Trischitta V, Consoli A, Sesti G. TRIB3 R84 variant is associated with impaired insulin-mediated nitric oxide production in human endothelial cells. *Arterioscler Thromb Vasc Biol.* 2008;28(7):1355-60.

- 206: Bergandi L, Silvagno F, Russo I, Riganti C, Anfossi G, Aldieri E, Ghigo D, Trovati M, Bosia A. Insulin stimulates glucose transport via nitric oxide/cyclic GMP pathway in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 2003;23(12):2215-21.
- 207: Chandra S, Mondal D, Agrawal KC. HIV-1 protease inhibitor induced oxidative stress suppresses glucose stimulated insulin release: protection with thymoquinone. *Exp Biol Med (Maywood).* 2009;234(4):442-53.
- 208: Mondal D, Liu K, Hamblin M, Lasky JA, Agrawal KC. Nelfinavir suppresses insulin signaling and nitric oxide production by human aortic endothelial cells: protective effects of thiazolidinediones. *Ochsner J.* 2013;13(1):76-90.
- 209: Parker RA, Flint OP, Mulvey R, Elosua C, Wang F, Fenderson W, Wang S, Yang WP, Noor MA. Endoplasmic reticulum stress links dyslipidemia to inhibition of proteasome activity and glucose transport by HIV protease inhibitors. *Mol Pharmacol.* 2005;67(6):1909-19.
- 210: Cheang WS, Tian XY, Wong WT, Lau CW, Lee SS, Chen ZY, Yao X, Wang N, Huang Y. Metformin protects endothelial function in diet-induced obese mice by inhibition of endoplasmic reticulum stress through 5' adenosine monophosphate-activated protein kinase-peroxisome proliferator-activated receptor δ pathway. *Arterioscler Thromb Vasc Biol.* 2014;34(4):830-6.
- 211: Zhou B, Li H, Liu J, Xu L, Guo Q, Zang W, Sun H, Wu S. Autophagic dysfunction is improved by intermittent administration of osteocalcin in obese mice. *Int J Obes (Lond).* 2016. doi: 10.1038/ijo.2016.1.
- 212: Camargo A, Meneses ME, Rangel-Zuñiga OA, Perez-Martinez P, Marin C, Delgado-Lista J, Paniagua JA, Tinahones FJ, Roche H, Malagon MM, Perez-Jimenez F, Lopez-Miranda J. Endoplasmic reticulum stress in adipose tissue determines postprandial lipoprotein metabolism in metabolic syndrome patients. *Mol Nutr Food Res.* 2013;57(12):2166-76.
- 213: Boden G, Cheung P, Kresge K, Homko C, Powers B, Ferrer L. Insulin resistance is associated with diminished endoplasmic reticulum stress responses in adipose tissue of healthy and diabetic subjects. *Diabetes.* 2014 Sep;63(9):2977-83.
- 214: Usui M, Yamaguchi S, Tanji Y, Tominaga R, Ishigaki Y, Fukumoto M, Katagiri H, Mori K, Oka Y, Ishihara H. Atf6 α -null mice are glucose intolerant due to pancreatic β -cell failure on a high-fat diet but partially resistant to diet-induced insulin resistance. *Metabolism.* 2012 Aug;61(8):1118-28.
- 215: Berisha SZ, Hsu J, Robinet P, Smith JD. Transcriptome analysis of genes regulated by cholesterol loading in two strains of mouse macrophages associates lysosome pathway and ER stress response with atherosclerosis susceptibility. *PLoS One.* 2013;8(5):e65003.
- 216: Ti Y, Xie GL, Wang ZH, Bi XL, Ding WY, Wang J, Jiang GH, Bu PL, Zhang Y, Zhong M, Zhang W. TRB3 gene silencing alleviates diabetic cardiomyopathy in a type 2 diabetic rat model. *Diabetes.* 2011;60(11):2963-74.

- 217: Zhang W, Liu J, Tian L, Liu Q, Fu Y, Garvey WT. TRIB3 mediates glucose-induced insulin resistance via a mechanism that requires the hexosamine biosynthetic pathway. *Diabetes*. 2013;62(12):4192-200.
- 218: Prudente S, Hribal ML, Flex E, Turchi F, Morini E, De Cosmo S, Bacci S, Tassi V, Cardellini M, Lauro R, Sesti G, Dallapiccola B, Trischitta V. The functional Q84R polymorphism of mammalian Tribbles homolog TRB3 is associated with insulin resistance and related cardiovascular risk in Caucasians from Italy. *Diabetes*. 2005;54(9):2807-11.
- 219: Prudente S, Scarpelli D, Chandalia M, Zhang YY, Morini E, Del Guerra S, Perticone F, Li R, Powers C, Andreozzi F, Marchetti P, Dallapiccola B, Abate N, Doria A, Sesti G, Trischitta V. The TRIB3 Q84R polymorphism and risk of early-onset type 2 diabetes. *J Clin Endocrinol Metab*. 2009;94(1):190-6.
- 220: Gong HP, Wang ZH, Jiang H, Fang NN, Li JS, Shang YY, Zhang Y, Zhong M, Zhang W. TRIB3 functional Q84R polymorphism is a risk factor for metabolic syndrome and carotid atherosclerosis. *Diabetes Care*. 2009;32(7):1311-3.
- 221: Cui AD, Gai NN, Zhang XH, Jia KZ, Yang YL, Song ZJ. Decreased serum obestatin consequent upon TRIB3 Q84R polymorphism exacerbates carotid atherosclerosis in subjects with metabolic syndrome. *Diabetol Metab Syndr*. 2012;4(1):52-60.
- 222: Formoso G, Di Tomo P, Andreozzi F, Succurro E, Di Silvestre S, Prudente S, Perticone F, Trischitta V, Sesti G, Pandolfi A, Consoli A. The TRIB3 R84 variant is associated with increased carotid intima-media thickness in vivo and with enhanced MAPK signalling in human endothelial cells. *Cardiovasc Res*. 2011;89(1):184-92.
- 223: Prudente S, Trischitta V. The TRIB3 Q84R polymorphism, insulin resistance and related metabolic alterations. *Biochem Soc Trans*. 2015;43(5):1108-11.
- 224: Geng T, Hu W, Broadwater MH, Snider JM, Bielawski J, Russo SB, Schwacke JH, Ross J, Cowart LA. Fatty acids differentially regulate insulin resistance through endoplasm reticulum stress-mediated induction of tribbles homologue 3: a potential link between dietary fat composition and the pathophysiological outcomes of obesity. *Diabetologia*. 2013;56(9):2078-87.
- 225: Wang YG, Shi M, Wang T, Shi T, Wei J, Wang N, Chen XM. Signal transduction mechanism of TRB3 in rats with non-alcoholic fatty liver disease. *World J Gastroenterol*. 2009;15(19):2329-35.
- 226: Wang W, Sun A, Lv W, Cheng J, Lv S, Liu X, Guan G, Liu G. TRB3, up-regulated in kidneys of rats with type1 diabetes, mediates extracellular matrix accumulation in vivo and in vitro. *Diabetes Res Clin Pract*. 2014;106(1):101-9.
- 227: Wang W, Cheng J, Sun A, Lv S, Liu H, Liu X, Guan G, Liu G. TRB3 mediates renal tubular cell apoptosis associated with proteinuria. *Clin Exp Med*. 2015;15(2):167-77.

- 228: Zhang W, Yang Z, Li X, Wen J, Zhang H, Wang S, Wang X, Zhou H, Fang W, Qin L, Su Q. The functional Q84R polymorphism of TRIB3 gene is associated with diabetic nephropathy in Chinese type 2 diabetic patients. *Gene*. 2015;555(2):357-61.
- 229: Oberkofler H, Pfeifenberger A, Soyal S, Felder T, Hahne P, Miller K, Krempler F, Patsch W. Aberrant hepatic TRIB3 gene expression in insulin-resistant obese humans. *Diabetologia*. 2010;53(9):1971-5.
- 230: Zhang X, Fu L, Zhang Q, Yan L, Ma Y, Tu B, Liu N, Qiao J. Association of TRB3 Q84R polymorphism with polycystic ovary syndrome in Chinese women. *Reprod Biol Endocrinol*. 2011;9:46-53.
- 231: Nakayama K, Lkhagvasuren M, Yanagisawa Y, Utsumi N, Kumada M, Gotoh T, Iwamoto S, Kagawa Y. Comparative study of polymorphisms on genes associated with lifestyle related diseases in Asian and Pacific populations. *Asia Pac J Public Health*. 2008;20 Suppl:173-9.
- 232: Wijekoon EP, Brosnan ME, Brosnan JT. Homocysteine metabolism in diabetes. *Biochem Soc Trans*. 2007;35(Pt 5):1175-9.
- 233: McCully KS. Homocysteine and the pathogenesis of atherosclerosis. *Expert Rev Clin Pharmacol*. 2015;8(2):211-9.
- 234: Facciorusso A. The influence of diabetes in the pathogenesis and the clinical course of hepatocellular carcinoma: recent findings and new perspectives. *Curr Diabetes Rev*. 2013;9(5):382-6.
- 235: Souza MR, Diniz Mde F, Medeiros-Filho JE, Araújo MS. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. *Arq Gastroenterol*. 2012;49(1):89-96.
- 236: Zerbini G, Gabellini D, Maestroni S, Maestroni A. Early renal dysfunctions in type 1 diabetes and pathogenesis of diabetic nephropathy. *J Nephrol*. 2007;20 Suppl 12:S19-22.
- 237: Vallon V, Komers R. Pathophysiology of the diabetic kidney. *Compr Physiol*. 2011;1(3):1175-232.
- 238: Morse E, Schroth J, You YH, Pizzo DP, Okada S, Ramachandrarao S, Vallon V, Sharma K, Cunard R. TRB3 is stimulated in diabetic kidneys, regulated by the ER stress marker CHOP, and is a suppressor of podocyte MCP-1. *Am J Physiol Renal Physiol*. 2010;299(5):F965-72.
- 238: Tripathi YB, Pandey V. Obesity and endoplasmic reticulum (ER) stresses. *Front Immunol*. 2012;3:240-47.
- 239: Khan S, Wang CH. ER stress in adipocytes and insulin resistance: mechanisms and significance (Review). *Mol Med Rep*. 2014;10(5):2234-40.
- 240: Scheuner D, Vander Mierde D, Song B, Flamez D, Creemers JW, Tsukamoto K, Ribick M, Schuit FC, Kaufman RJ. Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. *Nat Med*. 2005;11(7):757-64.

- 241: Mihai AD, Schröder M. Glucose starvation and hypoxia, but not the saturated fatty acid palmitic acid or cholesterol, activate the unfolded protein response in 3T3-F442A and 3T3-L1 adipocytes. *Adipocyte*. 2015;4(3):188-202.
- 242: Han J, Murthy R, Wood B, Song B, Wang S, Sun B, Malhi H, Kaufman RJ. ER stress signalling through eIF2 α and CHOP, but not IRE1 α , attenuates adipogenesis in mice. *Diabetologia*. 2013;56(4):911-24. doi: 10.1007/s00125-012-2809-5.
- 243: Araki E, Oyadomari S, Mori M. Impact of endoplasmic reticulum stress pathway on pancreatic beta-cells and diabetes mellitus. *Exp Biol Med (Maywood)*. 2003;228(10):1213-7.
- 244: Shirakawa J, Togashi Y, Sakamoto E, Kaji M, Tajima K, Orime K, Inoue H, Kubota N, Kadowaki T, Terauchi Y. Glucokinase activation ameliorates ER stress-induced apoptosis in pancreatic β -cells. *Diabetes*. 2013;62(10):3448-58.
- 245: Farmer SR. Regulation of PPAR γ activity during adipogenesis. *Int J Obes(Lond)*. 2005;29 Suppl 1:S13-6.
- 246: Bezy O, Vernochet C, Gesta S, Farmer SR, Kahn CR. TRB3 blocks adipocyte differentiation through the inhibition of C/EBP β transcriptional activity. *Mol Cell Biol*. 2007;27(19):6818-31.
- 247: Younce C, Kolattukudy P. MCP-1 induced protein promotes adipogenesis via oxidative stress, endoplasmic reticulum stress and autophagy. *Cell Physiol Biochem*. 2012;30(2):307-20.
- 248: Suh DH, Kim MK, Kim HS, Chung HH, Song YS. Unfolded protein response to autophagy as a promising druggable target for anticancer therapy. *Ann N Y Acad Sci*. 2012;1271:20-32.
- 249: Martinon F. Targeting endoplasmic reticulum signaling pathways in cancer. *Acta Oncol*. 2012;51(7):822-30.
- 250: Hou X, Liu Y, Liu H, Chen X, Liu M, Che H, Guo F, Wang C, Zhang D, Wu J, Chen X, Shen C, Li C, Peng F, Bi Y, Yang Z, Yang G, Ai J, Gao X, Zhao S. PERK silence inhibits glioma cell growth under low glucose stress by blockage of p-AKT and subsequent HK2's mitochondria translocation. *Sci Rep*. 2015;5:9065-74. doi:10.1038/srep09065.
- 251: Hurvitz SA, Kalous O, Conklin D, Desai AJ, Dering J, Anderson L, O'Brien NA, Kolarova T, Finn RS, Linnartz R, Chen D, Slamon DJ. In vitro activity of the mTOR inhibitor everolimus, in a large panel of breast cancer cell lines and analysis for predictors of response. *Breast Cancer Res Treat*. 2015;149(3):669-80.
- 252: Ojha R, Bhattacharyya S, Singh SK. Autophagy in Cancer Stem Cells: A Potential Link Between Chemoresistance, Recurrence, and Metastasis. *Biores Open Access*. 2015;4(1):97-108.
- 253: Saito Rde F, Tortelli TC Jr, Jacomassi MD, Otake AH, Chammas R. Emerging targets for combination therapy in melanomas. *FEBS Lett*. 2015;589(22):3438-48.

- 254: Blaustein M, Pérez-Munizaga D, Sánchez MA, Urrutia C, Grande A, Risso G, Srebrow A, Alfaro J, Colman-Lerner A. Modulation of the Akt pathway reveals a novel link with PERK/eIF2 α , which is relevant during hypoxia. *PLoS One*. 2013;8(7):e69668.
- 255: Borsi E, Terragna C, Brioli A, Tacchetti P, Martello M, Cavo M. Therapeutic targeting of hypoxia and hypoxia-inducible factor 1 α in multiple myeloma. *Transl Res*. 2015;165(6):641-50.
- 256: Niu Z, Wang M, Zhou L, Yao L, Liao Q, Zhao Y. Elevated GRP78 expression is associated with poor prognosis in patients with pancreatic cancer. *Sci Rep*. 2015;5:16067. doi: 10.1038/srep16067.
- 257: Matsuo K, Gray MJ, Yang DY, Srivastava SA, Tripathi PB, Sonoda LA, Yoo EJ, Dubeau L, Lee AS, Lin YG. The endoplasmic reticulum stress marker, glucose-regulated protein-78 (GRP78) in visceral adipocytes predicts endometrial cancer progression and patient survival. *Gynecol Oncol*. 2013;128(3):552-9.
- 258: Li H, Chen X, Gao Y, Wu J, Zeng F, Song F. XBP1 induces snail expression to promote epithelial-to-mesenchymal transition and invasion of breast cancer cells. *Cell Signal*. 2015;27(1):82-9.
- 259: Wu X, Xin Z, Zhang W, Zheng S, Wu J, Chen K, Wang H, Zhu X, Li Z, Duan Z, Li H, Liu Y. A missense polymorphism in ATF6 gene is associated with susceptibility to hepatocellular carcinoma probably by altering ATF6 level. *Int J Cancer*. 2014;135(1):61-8.
- 260: Capoccia BJ, Lennerz JK, Bredemeyer AJ, Klco JM, Frater JL, Mills JC. Transcription factor MIST1 in terminal differentiation of mouse and human plasma cells. *Physiol Genomics*. 2011;43(3):174-86.
- 261: Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, Solow-Cordero DE, Bouley DM, Offner F, Niwa M, Koong AC. Identification of an Ire1 α endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood*. 2011;117(4):1311-4.
- 262: López-Ayllón BD, de Castro-Carpeño J, Rodríguez C, Pernía O, Ibañez de Cáceres I, Belda-Iniesta C, Perona R, Sastre L. Biomarkers of erlotinib response in non-small cell lung cancer tumors that do not harbor the more common epidermal growth factor receptor mutations. *Int J Clin Exp Pathol*. 2015;8(3):2888-98.
- 263: Misra UK, Pizzo SV. Modulation of the unfolded protein response in prostate cancer cells by antibody-directed against the carboxyl-terminal domain of GRP78. *Apoptosis*. 2010;15(2):173-82.
- 264: Gunter JH, Sarkar PL, Lubik AA, Nelson CC. New players for advanced prostate cancer and the rationalisation of insulin-sensitising medication. *Int J Cell Biol*. 2013;2013:834684.
- 265: Lamoureux F, Thomas C, Yin MJ, Fazli L, Zoubeydi A, Gleave ME. Suppression of heat shock protein 27 using OGX-427 induces endoplasmic reticulum stress and potentiates heat shock protein 90 inhibitors to delay castrate-resistant prostate cancer. *Eur Urol*. 2014;66(1):145-55.

266: Xu J, Lv S, Qin Y, Shu F, Xu Y, Chen J, Xu BE, Sun X, Wu J. TRB3 interacts with CtIP and is overexpressed in certain cancers. *Biochim Biophys Acta*. 2007;1770(2):273-8.

X. Figure Legends

Figure-1: *Functional domains of TRIB3 protein.* (A) The N-terminal domain of TRIB3 contains a protein degradation motif (PEST) and a nuclear localization signal (NLS). The central domain contains the divergent kinase catalytic core. The C-terminal domain contains a MEK1 binding site and a COP1 binding site. (B) The ‘pseudokinase’ function of TRIB3 inhibits phosphorylation (activation) of AKT and suppresses the PI3K/AKT/mTOR axis. This function also inhibits I κ B phosphorylation and increases nuclear NF- κ B levels. The MEK1 binding motif inhibits numerous MAP-kinases and abrogates the downstream RAS/RAF/MEK/ERK axis. The COP1 motif interacts with E3 ubiquitin ligase to regulate proteasomal degradation of TRIB client proteins, e.g. transcription factors C/EBP α , PPAR γ and ATF-4.

Figure-2: *Exacerbated UPR causes ERS progression that induces TRIB3 expression.* Accumulation of unfolded proteins results in cytotoxicity, unless cellular homeostasis is restored via the unfolded protein response (UPR). The UPR increases ER chaperones, e.g. Grp78, Grp94, contents to restore normal ER function. Under ER-stress (ERS) conditions, ATF-6, IRE1 and PERK dissociate from Grp78 and activate multiple downstream pathways, which either enable cell homeostasis or progression of ERS. The PERK-eIF2 α pathway suppresses global protein synthesis, but upregulates both ATF4 and CHOP levels. Thus, prolonged ER-stress overwhelms UPR survival mechanisms to initiate pro-apoptotic pathways by activating the transcription factors (CHOP and ATF4) that enhance TRIB3 expression.

Figure-3: *TRIB3 accumulation tips the balance of cell survival and death.* The UPR induced transcription factors, ATF4 and CHOP increases TRIB3 expression. Under mild or transient ERS, TRIB3 acts via a negative feedback mechanism to inhibit ATF4 and CHOP, thereby promoting cell survival. However,

under severe or sustained ERS, continued expression of ATF4 and CHOP leads to the accumulation of TRIB3. Furthermore, TRIB3 mediated suppression of survival pathways and increased degradation of transcription factors promotes cellular dysfunctions and ultimately results in cell death.

Figure-4: *Multimodal actions of TRIB3 at the nexus of multiple signaling nodes.* TRIB3 inhibits AKT phosphorylation and suppresses the PI3K/AKT/mTOR pathway. TRIB3 associates with multiple MAP-Kinases and inhibits the RAS/RAF/MEK/ERK axis. TRIB3 increases ubiquitination of multiple client proteins, e.g. C/EBP α , PPAR γ , ATF4, and increases their proteasomal degradation. TRIB3 also activates Caspase-3 to increase apoptotic pathways. There are significant cross-talks between the UPR/ERS, PI3K/AKT/mTOR and RAS/RAF/MEK/ERK signaling pathways, and their effector proteins are also known to regulate both autophagy and lysosomal degradation of cellular constituents. TRIB3 is situated at the nexus of multiple signaling nodes and fine-tunes stress-inductive and stress-adaptive mechanisms.

Figure-5: *Effect of TRIB3 on insulin signaling and glycogen synthesis.* The ER-stress induced protein TRIB3 can inhibit insulin receptor (IR) signaling by suppressing AKT activation (phosphorylation). The insulin signaling cascade involves the activation of IR, followed by the activation of insulin receptor substrate (IRS1), phosphatidyl-inositol-kinase (PI3K) and PI3K dependent kinase (PDK1), which causes AKT phosphorylation. The activated AKT can then facilitate glucose transport via the mobilization of glucose transporter-4 (GLUT4) to the plasma membrane. Activated AKT also activates endothelial nitric oxide synthase enzyme (eNOS) and facilitates the production of nitric oxide (NO) for maintenance of vascular homeostasis. Activated AKT also increases glycogen synthesis and storage via the phosphorylation of glycogen synthase kinase (GSK3). Thus, the ‘pseudokinase’ function of TRIB3 disrupts multiple downstream effects of insulin signaling by inhibiting AKT phosphorylation.

Figure-6: *TRIB3 precariously balances homeostasis, metabolic dysfunction and cancer.* TRIB3 causes insulin resistance and facilitates the progression of metabolic syndrome associated manifestations like obesity, diabetes, hypertension and atherosclerosis. Changes in TRIB3 expression and/or subcellular localization can alter cellular signaling networks that lead to aggressive phenotype of cancer cells, e.g. proliferation, migration, survival mechanisms, pro-angiogenic effects and drug resistance. Therefore, TRIB3 is a common metabolic ‘switch’ that links health, metabolic dysfunction and cancer.

(A)

(B)

Figure-1

Figure-2

Figure-3

Figure-4

Figure-5

Figure-6