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Review Article

MICRORNA AND RNA BINDING PROTEINS: THE POSTTRANSCRIPTIONAL REGULATORS OF FOXO EXPRESSION

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ABSTRACT

FOXO (Forkhead box O) transcription factors, member of the forkhead family of proteins, a family which is characterized by the presence of 'forkhead box' a conserved DNA binding domain. Multiple environmental and other factors control FOXO activity by altering the various mechanisms of post-translational, transcriptional and post-transcriptional modifications. Regulation of FOXO expression at the posttranscriptional level came out as a new area of interest in controlling FOXO functions in normal and in cancerous cells. At posttranscriptional level, FOXO expression is regulated by microRNA and RNA binding proteins (HuR and QKI). MicroRNAs directly target multiple regions of the 3'-UTR of FOXO mRNA to down regulate its expression. Various miRNAs have been found to regulate expression of FOXO in different physiological and pathological conditions, including cancer. HuR overexpression in MDA-MB-231, breast cancer cell line results in stabilized FOXO1 mRNA and enhanced level of the FOXO1 factor. HuR-mediated up-regulation of FOXO1 causes apoptosis in cancer cells, upon exposure to a stressful stimulus. QKI has been found to reduce the stability of FOXO1 mRNA as well as to alter expression of FOXO1 in breast cancer cells. Collecting evidence suggests that this post-transcriptional level of regulation of FOXO expression is involved in reactions and adaptations to various stressful conditions. Alteration in FOXO posttranscriptional regulation is often linked to different disease conditions. Future research has to be done to resolve the complexities of posttranscriptional regulatory interactions that will create new knowledge about the novel pathways of disease processes and development of corresponding novel therapeutic molecule.

Keywords: FOXO, Posttranscriptional regulation, miRNA, Quaking, HuR

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INTRODUCTION

The forkhead or winged-helix family of transcription factors was named after the founding gene member in Drosophila (forkhead gene) [1]. FOXO (forkhead box O) transcription factor, member of the forkhead family of proteins, a family which is characterized by the presence of 'forkhead box' a conserved DNA binding domain [2]. There are four members in this conserved family, FOXO1 (also known as FKHR), FOXO3 (also known as FKHRL1), FOXO4 (also known as AFX1) and FOXO6, and are known as the subclasses of the forkhead family of transcription factors. Expression site of all these subclasses varies, FOXO1 and FOXO3 is expressed in almost all tissues. FOXO4 expression is higher in muscle, kidney, and colorectal tissue while FOXO6 is primarily expressed in the brain and liver [3, 4]. FOXO proteins regulate transcription in the cell nucleus and are involved in the transcription of genes that regulate diverse cellular processes including fuel metabolism, differentiation, proliferation, longevity, cell cycle progression, oxidative stress response, redox signalling and apoptosis [3, 5]. Recent studies have shown important tumour suppression activity of forkhead-BOX O transcription factors (FOXOs) by promoting cell cycle arrest, apoptosis and DNA damage repair [6]. The FOXO transcription factors are homologous to the *Caenorhabditis elegans* transcription factor DAF-16 (abnormal DAuer formation-16), which regulates lifespan downstream of the C. elegans insulin receptor DAF-2 [7]. Varieties of external stimuli, such nutrients, cytokines, insulin, insulin-like growth factor I (IGF-I), and oxidative stress regulate FOXO transcription factors. These environmental factors control FOXO activity by altering the various mechanisms of post-translational modifications of FOXO, such as phosphorylation, acetylation, ubiquitination, and methylation, which in turn regulate subcellular localization, protein levels, DNA-binding properties, and transcriptional activity [8]. Together with the post-translational regulation of FOXOs, these are also regulated at transcriptional as well as post-transcriptional level. Less is known about the regulation of FOXO mRNA expression. At transcription level, some transcription factors including certain E2F isoforms, p53 and FOXOs (specifically, FOXO3 regulating FOXO1) are shown to control its expressions. The transcription factor E2F1 was shown to induce FOXO1 and FOXO3 expression, and FOXO3 up-regulates FOXO1 in the eye [5, 9]. Posttranscriptional regulation of FOXO expression emerges as a nifty level of complication in controlling FOXO functions in both normal and cancer cells. Four different mechanisms have been identified so far: (i) the RNA-binding protein (RBP) Human antigen R (HuR), stabilizes FOXO mRNA (ii) the RBP Quaking (QKI) decreases FOXO mRNA durability (iii) the FOXO1a 3'UTR may work as a competing endogenous RNA (ceRNA), and (iv) myriad microRNAs (miRNA) have been described that direct FOXO transcripts [10]. In this review, we will focus on the posttranscriptional regulation of FOXOs expression by miRNAs and by RBP, including HuR and QKI.

Search criteria

In this review, articles were included from various electronic databases: Medline, Embase, Science Direct, PubMed, Scopus, Web of Science, Scirus, and Google Scholar by using several keywords for search: posttranscriptional regulation, posttranslational modification, FOXO, RNA binding proteins, quaking, HuR, miRNAs, cancer, target genes, transcription factors. The range of years used as the filter was from 2010-2017. Mostly latest journals were included. EndNote was used for citation and bibliography. CAS Source Index (CASSI) Search tool was used for the abbreviation of journals. 1 checker was used for grammar and spelling checking. Paint 3D and Inkscape were used for drawing of figures.

Cellular functions of FOXOs

Diverse cellular functions of FOXOs are known (fig. 2). Inside the nucleus, FOXO proteins via the fork headbox, a 110 amino-acid region located in the central part of the molecule bind to DNA as monomers (fig. 1). When FOXO factors bind to DNA molecule inside the nucleus typically it acts as potent transcriptional activators. Thus, depending on the promoter context and extracellular conditions, this family of transcription factors, may activate or repress transcription [2, 6, 11]. FOXO proteins encourage cell cycle arrest at the G1/S boundary [12] by utilizing two ways, by suppressing cell cycles activators (cyclin D1/D2) and by up regulating cell cycle inhibitors (p21 and p27KIP1) [6]. Target genes such as Cdk inhibitor p27KIP1 and the Rb family member p130

intercede FOXO-mediated cell cycle arrest [12, 13]. In the presence of transforming growth factor beta (TGFb), it also binds to the promoter region of cell cycle inhibitor target gene p21, and induce cell cycle arrest at the G1/S transition [14]. FOXO factors by suppressing the expression of cell cycle positive regulators cyclin D1 and D2 can also encourage cell cycle arrest [15, 16]. There are two FOXO3 target genes, Cyclin G2 and growth arrest and DNA damage-inducible protein 45 (GADD45) that may mediate FOXOs' cell cycle arrest at the G2/M boundary [17]. Expression of active forms of FOXO factors up regulates several genes that are involved in DNA repair [15, 18]. Fibroblasts having a deficiency of GADD45 gene have shown reduced FOXO3-induced DNA repair which signifies GADD45 may facilitate part of FOXO3-induced DNA repair [18]. FOXO factors have been shown involved in up regulating the free radical scavenging enzymes, such as Mn superoxide dismutase (MnSOD) and catalase that cause detoxification of free radicals [15, 18, 19].

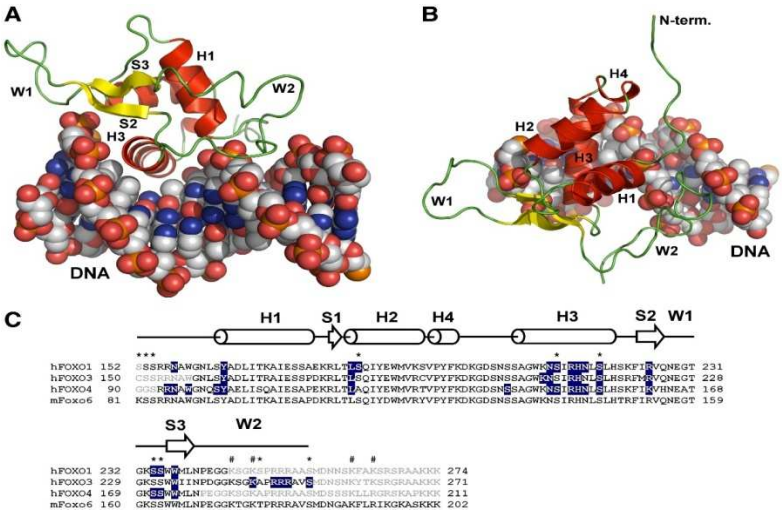

Fig. 1: The crystal structure of the FOXO-DNA complex [20], (A) Ribbon represents forkhead domain of FOXO and the spheres represent DNA. Labelling of secondary structure elements are done according to the nomenclature typical for the winged–helix motif (B) 90 ° rotated structure towards the viewer relative to A around the horizontal axis. (C) Sequence alignment of FOXO forkhead domains. At the top Secondary-structure elements are indicated. Blue color indicates residues involved in protein–DNA contacts. Grey indicates residues that are disordered or missing in the corresponding FOXO–DNA structures. Symbol (*) signifies phosphorylation sites. Symbol (#) signifies acetylation and/or mono ubiquitination sites

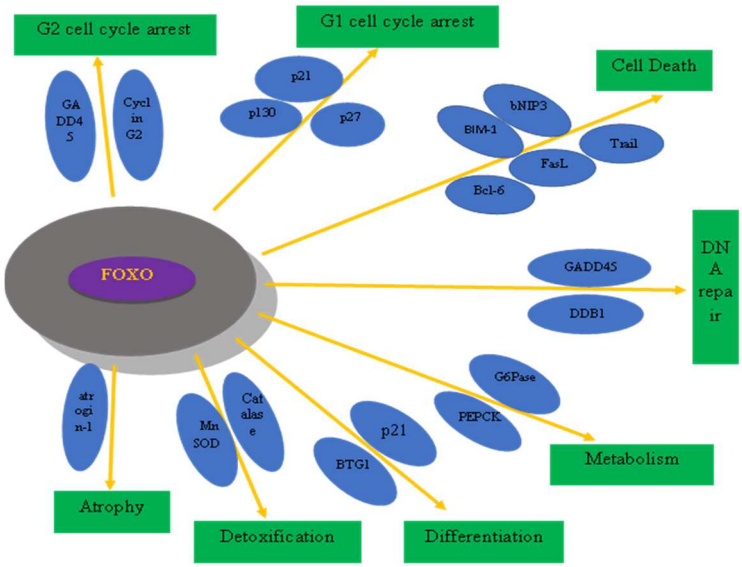

Fig. 2: Cellular functions of various FOXO target genes [12-33], FOXO factors induce transcription of variety of target genes that show various cellular functions. GADD45: growth arrest and DNA damage-inducible protein 45; DDB1: damage-specific DNA-binding protein 1; p21: cyclin-dependent kinase inhibitor 1A; p27: cyclin-dependent kinase inhibitor 1B; p130: Rb family member; BIM-1 and bNIP3: Bcl-2 proapoptotic family member; FasL: Fas ligand; Trail: Cytokine; Bcl-6: Repressor of BCL-XL; G6Pase: glucose-6-phosphatase; PEPCK: phosphoenolpyruvate carboxykinase; BTG-1: B-cell translocation gene 1; MnSOD: manganese superoxide dismutase

Involvement of FOXO factors in inhibiting and elevating cell differentiation depends on the cell type and the type of FOXO isoform. FOXO3 by inducing B-cell translocation gene-1 (BTG1) indicated to promote erythroid differentiation. [21]. In *in vitro* differentiation assays, FOXO1 expression has been shown to inhibit differentiation in adipocytes and myoblasts. Inhibition mechanisms are not fully understood but it is assumed that in adipocytes, expression of cycle inhibitor p21CIP1 get directly up regulated by FOXO1, without altering p27KIP1 or the C/EBP dimerization partner Chop10 [22, 23] while FOXO nuclear forms expression induces cell death in neurons and in lymphocytes [24]. Serine/threonine-specific protein kinase (Akt) and serum and glucocorticoid-inducible kinases (SGK) encourage cell

growth by sequestering FOXO factors away from death genes. FOXO target genes proapoptotic Bcl-2 family member BIM, and bNIP3 (Microarray analysis identified) mediate apoptosis [18]. Addition to this, the expression of a prosurvival or anti-apoptotic Bcl2 family member BclxL, get down regulated indirectly by FOXO4 by up regulation of Bcl-6, a transcriptional suppressor. Thus, apoptosis caused by FOXO involves modulation of the ratio of pro-death and prosurvival members of the Bcl-2 family [25]. FOXO proteins also found to cause apoptosis through stimulation of death cytokines, such as Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) [26, 27]. The ability of FOXO factors to induce apoptosis through triggering death pathways in neighbouring cells may get enhanced by FasL, TRAIL, and other death cytokines [24, 28]. FOXO3 expression induces atrophy in fully grown cardiac and skeletal muscle cells. FOXO causes muscular atrophy by a decrease in cell size rather than apoptosis. This reduction in cell size occurs due to FOXO dependent increase in the gene encoding atrogin-1 (a muscle-specific ubiquitin ligase, enhances protein degradation and muscle atrophy) that causes a reduction in global protein levels [29-31]. Thus, the Akt signalling pathway works to enhance cell size by activating mTOR signalling pathway and suppressing FOXO activity through a simultaneously enhancement in protein production and a decline in protein degradation [32]. Genes controlling glucose metabolism also get upregulated by FOXO proteins. FOXO factors upregulate glucose 6 phosphatases (G6Pase), an enzyme responsible for converting glucose 6 phosphate to glucose, and phosphoenolpyruvate carboxykinase (PEPCK), an enzyme that converts oxaloacetate to phosphoenol-pyruvate (PEP) [23, 29, 33].

Regulation of FOXO activity

Posttranslational modification

Transcriptional activity of FOXO is regulated by a complex array of posttranslational modifications (fig. 3), either by activating or inactivating. They act by modulating nuclear import and export steps, alters the DNA complexing affinity, and modify the way of transcriptional activity towards a specific target. FOXOs bear four functionally distinct motifs which include a forkhead, nuclear localization, nuclear export, and transactivation domains that share significant sequence homology. FOXO factors subject to various post-translational modifications including phosphorylation, acetylation, ubiquitination, and methylation, which are closely related to their activity [34]. In turn, various FOXO activity including DNA binding, transactivation activity, FOXO subcellular localization and/or FOXO stability/degradation as well as complexation with transcriptional coregulators get affected by these modifications [5]. FOXOs undergo phosphorylation by various protein kinases at different sites due to its substrates nature for kinases that leads to the alteration of their DNA-binding, subcellular localization, and protein stability. FOXOs activity majorly regulated by phosphoinositide 3 kinase (PI3/K) pathway. Akt and SGK are important downstream elements of PI3/K signalling [35]. RXRXXS/T is the motifs for substrate phosphorylation which is recognized by both AKT and SGK and are responsible for phosphorylation of FOXO at Thr32, Ser253 and Ser315, 3 key residues (of FOXO3). Phosphorylation of FOXO at these residues leads to its association with 14-3-3 proteins that causes its export from the nucleus, where it becomes inactive transcriptionally [24, 36]. Mammalian sterile 20-like kinase (MST), interacts with FOXO3 upon oxidative stress and phosphorylates Ser207 within its forkhead domain. Binding of FOXO3 to14-3-3 proteins get disrupted upon MST1-mediated phosphorylation, that facilitates its accumulation to nucleus hence, expression of downstream pro-apoptotic genes gets induced that intern promotes neuronal cell death [37]. Cyclin-Dependent Kinase 1 (CDK1) is involved in cell cycle progression, predominantly at G2/M transition. It phosphorylates FOXO at Ser249 in the forkhead domain. In contrary to the PI3K/AKT pathway, phosphorylation by CDK1 at Ser249 causes disruption of binding between FOXO and 14-3-3 protein, thus leading to its nuclear accumulation [38]. Human FOXO3 gets phosphorylated by AMP-activated protein kinase (AMPK) at six regulatory locations. AMPK phosphorylation causes activation of FOXO3 transcriptional activity [89] without altering FOXO3 subcellular accumulation [39]. Extracellular signal-regulated kinases (ERK) causes phosphorylation of FOXO3 at Ser294, Ser344, and Ser425, that leads to its nuclear exclusion. Phosphorylation and nuclear exclusion of FOXO3 at these sites results in enhanced interaction with the ubiquitin E3-ligase, Mouse double minute 2 homolog (MDM2) which renders it unstable [40]. Inhibitor of kappa B kinase (IKK) activation has been shown to promote phosphorylation of FOXO3 at Ser644 residue. Phosphorylation of this residue causes nuclear exclusion and further degradation of FOXO3 factors. IKK and ERK both are found to act as oncogenes in carcinogenesis, which supports the FOXO factors hypothesis of tumor suppressors [41]. Acetylation of FOXO affects its activity that involves a complex balance between histone deacetylases (HDACs) including Silent Information Regulators (SIRT1) and (SIRT2) and the CREB Binding Protein (CBP), Histone Acetyl Transferases (HATs) and p300. Coactivators CBP/p300 histone acetyltransferase activity could increase transcription activity of FOXO by their histones acetylation ability indirectly and predominantly remodel chromatin. Opposite to it, FOXO (particularly FOXO1) acetylation has been found to attenuates its DNA binding ability and the increment of phosphorylation of FOXO1 level [42, 43]. FOXOs ubiquitination occurs in two ways, by polyubiquitination and by monoubiquitination. The ubiquitin-proteasome system (UPS) plays a crucial function in the degradation of FOXO proteins. Various proteases including a ubiquitin-conjugating enzyme (UBC, E2), ubiquitin-activating enzyme (E1), and ubiquitin ligase (E3) MDM2 [44] are found to involved in polyubiquitination. These enzymes induce degradation of FOXO3 by binding to it, through polyubiquitination that requires ERK [40]. Ser256 phosphorylated FOXO1can be recognized by Skp2, another E3 ligase which degrades it by polyubiquitination [45]. MDM2 induces monoubiquitination of FOXO4 upon oxidative stress [46]. Monoubiquitination causes nuclear localization of FOXO and hence, increases FOXO-dependent transcriptional activity. Methylation of FOXOs is mainly done by protein arginine methyltransferases (PRMT1) which methylate's primarily at Arg248 and Arg250 residues of FOXO1 that are conserved within the consensus Akt phosphorylation motif. Akt-induced Ser253 residue phosphorylation of FOXO1 get directly inhibited by this arginine methylation, which in turn causes its enhanced nuclear accumulation and subsequent inhibition of its degradation. PRMT1 decreased level hence, has shown nuclear exclusion of FOXO1 and further degradation [47, 48].

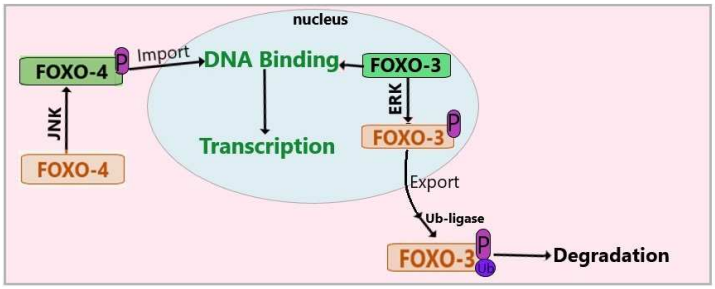

Fig. 3: Posttranslational modification of FOXOs [5], posttranslational modification affects FOXOs activity, their subcellular localization and stability. This fig. shows consequences of FOXO phosphorylation: nuclear import and export. JNK causes phosphorylation of FOXO4 that results in its nuclear import, increased DNA binding and further transcription while phosphorylation of FOXO3 by ERK causes its nuclear export and ubiquitination leading to its proteasomal degradation. (Red: inactive; green: active FOXO form)

Transcriptional modification

Transcription of the FOXO genes also affects FOXO level. E2F, p53 and FOXO3 itself are the major transcriptional regulators of expression of FOXOs (fig. 4) [10]. E2F-1 is a transcription factor which is involved in cell growth and apoptosis on which the level of FOXO1 and FOXO3 mRNA depends. E2F-1interacts to the specific promoter region of FOXO1 and FOXO3 [49]. This shows that these FOXO genes to be transcriptional targets of E2F-1 [50].

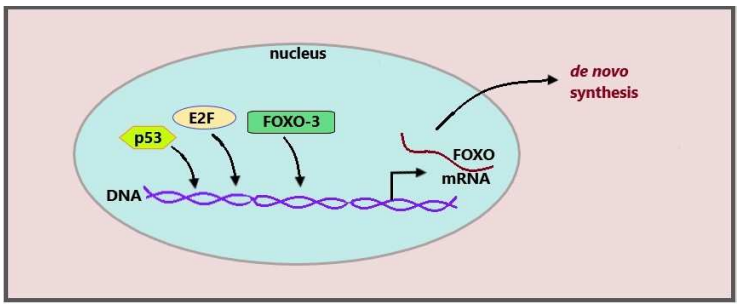

Fig. 4: Regulation of FOXO expression at the transcriptional level [5], transcription factors encompassing E2F isoforms, p53 and FOXOs (specifically, FOXO3 regulating FOXO1 expression) controlling FOXO expression

Post-transcriptional regulation of FOXO expression

Regulation of FOXO expression at posttranscriptional level (fig. 5), came out as a new level of challenge in regulating functions of FOXO in both normal and cancerous cells.

Three different mechanisms are known so far that controls FOXO expression at post-transcriptional level: (i) distinct miRNAs that target transcription factors(ii) HuR, an RBP that enhances FOXO-mRNA stability, (iii) QKI, an RBP that decreases FOXO mRNA stability [10].

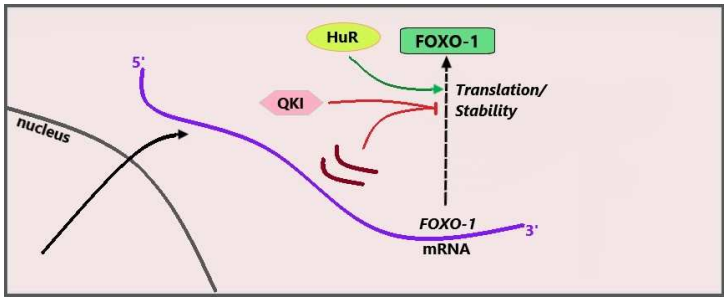

Fig. 5: Posttranscriptional level of regulation of FOXO activity and expression [5]. FOXO mRNA translation and activity are affected by miRNAs (bright red) and RNA-binding proteins such as HuR and quaking. miRNA and Quaking abolishes translation activity while HuR promotes it

Regulation of FOXO expression by miRNAs

MiRNA, a novel class of small RNA molecule, has been found to involve in the post-transcriptional regulation of thousands of mRNA that results in reduced protein expression of target genes [51]. It was discovered by Victor Ambros and had been developed since 2000 [86, 87]. MiRNAs are single-stranded RNA molecules which are having little ~21–25 nucleotides that negatively regulates expression of a target gene by interacting with the 3′-UTR of a target mRNA which results in either degradation or translational inhibition of the transcript. Interestingly, some miRNAs have multiple targets, and some others can be targeted by several miRNAs. Approximately 52% of miRNA genes are located the intergenic area, 40% are within introns, and 8% are within exons [88]. According to recent findings, a variety of miRNAs works in association to fine-tune protein expression on a global level [52, 53]. Addition to this, mice with microRNA knockout genes shows a variety of serious phenotypes including severe immune deficiency [54] and stress-induced heart defects [55]. These studies have focused on the crucial functions of these genes in tissue homeostasis and disease [56]. Various findings have indicated that out of three FOXO family members which are ubiquitously expressed, FOXO1 get down-regulated selectively in breast cancer in contrary to normal tissues. They also described that many miRNAs (miR-27a, miR-96, and miR-182), previously has been implicated in oncogenesis, regulate the expression of FOXO1. These microRNAs directly target multiple regions of the 3′-UTR of mRNA to downregulate endogenous expression of FOXO1. It has been shown by Stittrich *et al.* (2010) that IL-2/IL-2 receptor-dependent signalling induces stimulation of STAT5 or sp1 a transcription factor. Binding of activated STAT5 to the miR-182 gene regulatory region resulting in up to 200-fold upregulation in its expression. In turn, the formation of FOXO1 factor is reduced by Mature miR-182 which interacts with a specific site in the 3-UTR of its mRNA [57, 58]. Inhibition of these microRNAs has shown restoration of FOXO1 expression. It has been recently found by Gheysarzadeh *et al.* (2015) that, the STAT5 ↑/miR-182↑/FOXO1↓axis under conditions of oxidative stress can be changed to rapidly up-regulate FOXO1 activity (STAT5↓/miR-182↓/FOXO1↑) [59]. FOXO1 expression restoration in MCF-7 (Michigan Cancer Foundation-7) cells have shown decreased cell number, reduced cell cycle traverse, and increased cell death. Further enhanced effects were produced when overexpression of a constitutively active mutant FOXO1 factor was done. In addition, miRNA down-regulation anti-proliferative effects get blocked by FOXO1 restoration suppression by siRNA, hence functionally linking expression of miRNA, FOXO1, and cell proliferation and/or viability. These studies signifies that antisense targeting of miRNAs including, miR-27a, miR-96, and miR-182 together with focusing on miRNA and FOXO1 levels may have therapeutic as well as prognostic value in breast cancer treatment [60]. miR-155 also targets the same FOXO3 mRNA thereby, promotes oxidative stress in pancreatic cancer [61]. In addition, a polymorphism in the 3′-UTR of human FOXO1 mRNA, a miR-137-binding site has been found to reduce hereditary susceptibility to hepatocellular carcinoma [62]. Various miRNAs have been found to regulate expression of FOXO (table 1) [5].

Table 1: A list of miRNAs indicated to target FOXO mRNA [5]

miRNA	Target mRNA (FOXO isoform)	Target cell/tissue or biological process (organism)
miR-9	FOXO1	Breast cancer cells (Hs),
	FOXO1	Neural stem cell differentiation (Mm)
	FOXO1 and FOXO3	Haematopoietic cells (Hs and Mm)
miR-27a	FOXO1	Renal cell carcinoma (Hs)
	FOXO1	Liver cancer (Hs and Mm)
	FOXO3	Glioblastoma (Hs)
miR-96	FOXO1	Bladder cancer (Hs)
	FOXO1	Prostate cancer (Hs)
	FOXO1 and FOXO3	HepG2 hepatoma cells (Hs)
	FOXO3	Breast cancer (Hs)
	FOXO3	Idiopathic pulmonary fibrosis fibroblasts (Hs)
	FOXO3	Non-small cell lung cancer (Hs)
	FOXO3	Prostate cancer (Hs)
miR-107	FOXO1	Gastric cancer cells (Hs)
miR-126	FOXO3	Vascular smooth muscle cells (Hs and Mm)
miR-137	FOXO1	Hepatocellular carcinoma (Hs)
miR-155	FOXO3	Breast cancer (Hs)
	FOXO3	Hypoxic lung cancer cells (Hs)
	FOXO3	Multifunctional Treg cell line (Hs)
	FOXO3	Vascular stem cell niche in bone marrow (Hs)
miR-182	FOXO1	Activated helper T-lymphocytes (Mm and Hs)
	FOXO1	Osteoblasts and skeletogenesis (Mm and Dr)
	FOXO1 and FOXO3	Colon cancer African and Caucasian Americans (Hs)
	FOXO3	Lung cancer (Hs)
	FOXO3	Melanoma metastasis (Hs and Mm)
	FOXO3	Ovarian cancer cells–orthotopic xenografts (Hs)
	FOXO3	Skeletal muscle (Mm and Rn)
miR-183	FOXO1	Human-specific miR-183 target site (Hs)
	FOXO1	Non-small cell lung cancer cells (Hs)
miR-196a	FOXO1	Cervical cancer (Hs)

Hs: human; Mm: mouse; Rn: rat; Bt: bovine; Dr: zebrafish

Regulation of FOXO expression by RBP

HuR

TTR-RBPs family the mammalian embryonic lethal abnormal vision in Drosophila (Hu/elav) comprises of the primarily neuronal proteins including HuB, HuC, and HuD, and ubiquitous HuR (HuA) [63]. HuR, a regulatory protein that binds with the AU-rich element (ARE) and interacts with a various mRNAs that are involved in a myriad of cellular functions including, cell division, differentiation, cell survival and immune response. It has mRNA-stabilizing or translation promoting activity. The RNA binding protein HuR has the ability to sense and reacts to stressful stimuli which leads to its accumulation in the cytosol. There are various stressful stimuli to which HuR responds including, hydrogen peroxide [64, 65], or stimuli that generate ROS–including UV radiation [65, 66], arsenite exposure, or tert-butyl hydroquinone [67, 68]. These stimuli work by activating p38MAPK pathway, which stimulates phosphorylation of HuR directly or indirectly (e. g., via MAPK-activated kinase-2, MK2). This phosphorylation, in turn, causes stress-induced accumulation of HuR into the cytosol and increases its mRNA stabilizing activity [69]. Additionally, it was hypothesized that HuR itself works as a redox sensor. Homodimerization of HuR is essential for its full activity. For homodimerization cysteine residue in the first of the three HuR RNA recognition motifs was found to be important. These studies suggest that HuR homodimer formation may get affected due to the ability of this cysteine to respond towards oxidative stress which results in an elevated HuR activity due to homodimerization [5, 10, 70]. Various important medical conditions have been associated with an abnormality in the HuR expression levels or subcellular localization, including, atherosclerosis, tissue ischemia, pathologic inflammation, and, most promptly, tumor formation, growth, and metastasis [71-73]. However, in clinical trials, it has been found that there is a correlation exists between enhanced HuR expression levels and cytoplasmic expression pattern with malignant phenotype and poor patient prognosis in different types of cancer [74]. Bioinformatic prediction identified that FOXO1 is an important target of HuR as the 3'-UTR of FOXO1 is equipped with HuR binding motifs [75]. Li *et al.* (2013) in his studies have characterized two AREs present in the 3-UTR of human FOXO1 mRNA which is recognized by HuR. It has been found that HuR overexpression in M. D. Anderson-Metastatic Breast-231 (MDA-MB-231), breast cancer cell line results in stabilized FOXO1 mRNA and enhanced level of FOXO1 factor. Additionally, up-regulation of endogenous HuR through induced stress by using 5-fluorouracil (5-FU) also results in an enhanced level of cellular FOXO1 protein. SiRNA-induced knockdown of endogenous HuR caused complete inhibition of 5-FU induced apoptosis and it was restored by ectopic FOXO1 overexpression in the presence of HuR-siRNA. By considering these data it indicates that HuR-mediated up-regulation of FOXO1 causes apoptosis in cancer cells, upon exposure to a stressful stimulus (fig. 6). Due to this reason, modulation in the expression of FOXO1 has been indicated in the development of new therapeutic molecule for the treatment of various types of cancer [75].

QKI

QKI, encoded by the quaking (qk) locus is a KH-type of RBP [76]. QKI have three known isoforms, namely QKI-5, QKI-6 and QKI-7. Only QKI-5 is the nuclear isoform which shows nucleus-cytoplasmic shuttling i.e., it shuttles between the nucleus and the cytoplasm, while QKI-6 and QKI-7 are remain accumulated in the cytoplasm [77, 78]. QKI, an RBP binds to cis-elements within the 3'-UTR of target genes and regulates post-transcriptionally the stability of mRNA, mRNA splicing, translation efficiency or RNA transportation [79]. QKI also found to involved in the posttranscriptional regulation of FOXO1 [80, 81]. QKI is found to be essential for myelination during postnatal development as well as for embryogenesis. It has the ability to coordinates with differentiation and cell cycle and also it acts as a tumour suppressor [82]. It has been demonstrated by Yu *et al.* (2014) that QKI interacts with three QKI response elements (QRE) located within 3'-UTR of FOXO1. QKI has been found to reduce the stability of FOXO1 mRNA as well as to alter expression of FOXO1

in breast cancer cells: QKI knockdown by siRNA resulted in up-regulation of FOXO1 mRNA, while overexpression of QKI resulted in down-regulation of FOXO1. Significantly, proliferation inhibition by 5-FU treatment or all-trans-retinoic acid (ATRA) resulted in QKI down-regulation, up-regulation of FOXO1 expression and up-regulation of luciferase activity in the FOXO1 3'UTR assay [81]. Yu *et al.* (2014) based on these studies indicated that QKI targeting may provide a new insight into the development of novel therapeutic molecule for the treatment of various types of breast cancers.

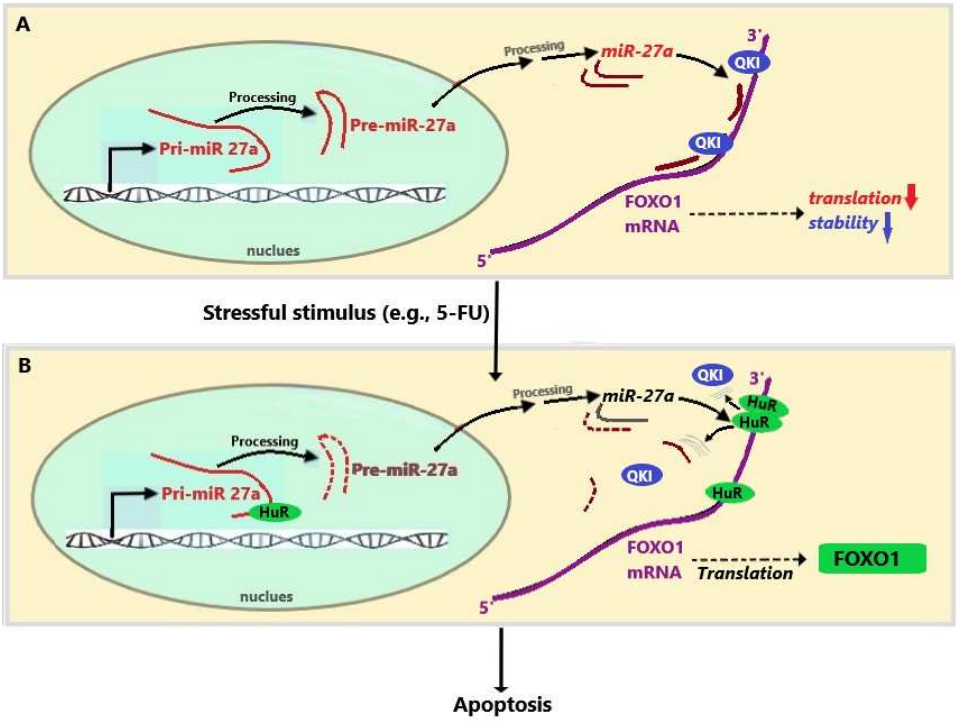

Fig. 6: Hypothesized interactions between HuR, quaking and miRNAs upon stressful stimulus in breast cancer cells [5], (A) fig. shows that, quaking (QKI) along with miR-27a, decreases translation and stability of FOXO1 mRNA. (B) 5-fluorouracil (5-FU) exposure creates a stressful condition that results in up-regulation of HuR and subsequently its cytosolic accumulation; simultaneously, QKI get down-regulated. HuR binds to FOXO1 mRNA, which leads to dissociation of miRNA and QKI, resulting in stabilization of mRNA and its translation up-regulation. Up-regulation of FOXO1 factor, in turn, induces apoptosis in breast cancer cells

Recent trends in post-transcriptional regulation of FOXOs

Recent studies are focusing primarily on post-transcriptional regulation of FOXO expression in various pathophysiological conditions. More recently the effects of different miRNAs on level of FOXO in various types of cancer has been of interest (see table 1 for details) [5]. It has been recently found by Gheysarzadeh *et al.* (2015) that, the STAT5 ↑/miR-182↑/FOXO1↓axis under the conditions of oxidative stress can be changed to rapidly up-regulate FOXO1 activity (STAT5↓/miR-182↓/FOXO1↑). In turn, this led to an enhanced synthesis of the pro-apoptotic FOXO1 target gene products including Bcl-2-interacting mediator of cell death (Bim), Bcl-2-associated X protein and to caspase-3 activation [59]. Studies by Guo *et al.* (2014) demonstrate that the regulatory axis QKI↑/FOXO1↓ may contribute a crucial role in ischemic heart injury susceptibility [80]. Studies by Mehta *et al.* (2015) showed that in ageing haematopoietic stem cell (HSCs) the cluster of miR-132/miR-212 is being responsible for controlling FOXO3 expression, which in turn ensures balanced HSC survival, maintenance and function [83]. Recent study by Zhao *et al.* (2014) has found that mice with ovarian cancer cells (SKOV3) treated with curcumin has shown miR-9 (Tumor suppressor gene) Overexpression resulting in enhanced cytotoxicity [84]. Dahmke *et al.* (2013) identified that mice suffering from B78H1 murine melanoma administered with curcumin diet revealed huge overexpression of the Tumor suppressor murine miRNA mmu-miR-205-5p (135-fold higher) and tumor regression. [85].

Future perspectives of post-transcriptional regulation of FOXOs

From the viewpoint of an emerging complexity of interactions between miRNAs and RBPs (QKI and HuR) in the wide range of posttranscriptional regulation of the FOXO transcripts indicated to impact multiple aspects of the survival and death of cells. However, most of the studies about these till date has been done are not satisfactory. Yu *et al.* (2014) studies indicated that QKI targeting may provide a new insight into the development of novel therapeutic molecule for the treatment of various types of breast cancers. Li *et al.* (2013) suggest that modulation in the expression of FOXO1 may be indicated in the development of new therapeutic molecule for the treatment of various types of cancer. Guttilla *et al.* (2009) studies signifies that antisense targeting of microRNAs including, miR-27a, miR-96, and miR-182 together with focusing on microRNA and FOXO1 levels may have therapeutic as well as prognostic value in breast cancer treatment. Urbánek *et al.* (2016) suggests that despite of so much studies being done many questions are arising focusing post-transcriptional regulation of FOXO expression that has to be resolved in future. For example, do miRNAs and QKI cooperate in downregulating FOXO1 protein? How does HuR abolish the inhibitory effect of miRNAs/QKI on FOXO1 expression? Does HuR suppress miR-27a biogenesis? Can HuR and QKI exert an inhibitory (rather than stimulatory) and stimulatory (rather than attenuating) action, respectively, on FOXO expression under certain physiological conditions? Are there other RNA-binding regulatory proteins that control or modulate FOXO expression post-transcriptionally?

CONCLUSION

Regulation of expression of FOXO at posttranscriptional level came out as a new area of interest in controlling FOXO functions in normal and in cancerous cells. So far various mechanisms have been determined that regulate FOXO expression at a posttranscriptional level which include miRNAs and RBPs (QKI and HuR). Various

miRNAs have been found to regulate expression of FOXO in myriads of physiological and pathological conditions, including cancer (table 1) in different types of cell and tissues. Many miRNAs (miR-27a, miR-96, and miR-182), previously been implicated in oncogenesis, regulate the expression of FOXO1. These microRNAs directly target multiple regions of the 3-UTR of mRNA to downregulate endogenous expression of FOXO1. Inhibition of these microRNAs has shown restoration of FOXO1 expression. These studies signify that antisense targeting of microRNAs including, miR-27a, miR-96, and miR-182 together with focusing on microRNA and FOXO1 levels may have therapeutic as well as prognostic value in breast cancer treatment. HuR overexpression in MDA-MB-231, breast cancer cell line results in stabilized FOXO1 mRNA and enhanced level of FOXO1factor. Available data suggest that HuR-mediated up-regulation of FOXO1 causes apoptosis in cancer cells, upon exposure to a stressful stimulus. Due to this reason, modulation in the expression of FOXO1 has been indicated in the development of new therapeutic molecule for the treatment of various types of cancer. QKI has been found to reduce the stability of FOXO1 mRNA as well as to alter expression of FOXO1 in breast cancer cells, hence QKI targeting may provide a new insight into the development of novel therapeutic molecule for the treatment of various types of breast cancers. By collecting all evidence, one can conclude that this post-transcriptional level of regulation of FOXO expression is involved in reactions and adaptations to various stressful stimuli. Alteration in FOXO posttranscriptional regulation is often linked to different disease conditions. Future research has to be done to resolve the complexities of posttranscriptional regulatory interactions that will create new knowledge about the novel pathways of disease processes and development of corresponding novel therapeutic molecules.

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CONFLICT OF INTERESTS

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