

Review Article

Mechanistic insights into the role of FOXO in diabetic retinopathy

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Received May 20, 2021; Accepted April 28, 2022; Epub June 15, 2022; Published June 30, 2022

Abstract: Diabetes mellitus (DM), a metabolic disorder characterized by insulin-deficiency or insulin-resistant conditions. The foremost microvascular complication of diabetes is diabetic retinopathy (DR). This is a multifaceted ailment mainly caused by the enduring adverse effects of hyperglycaemia. Inflammation, oxidative stress, and advanced glycation products (AGES) are part and parcel of DR pathogenesis. In regulating many cellular and biological processes, the family of fork-head transcription factors plays a key role. The current review highlights that FOXO is a requisite regulator of pathways intricate in diabetic retinopathy on account of its effect on microvascular cells inflammatory and apoptotic gene expression, and FOXO also has the foremost province in regulating cell cycle, proliferation, apoptosis, and metabolism. Blockage of insulin turns into an exaggerated level of glucose in the bloodstream and can upshot into the exaggerated triggering of FOXO1, which can ultimately uplift the production of several factors of apoptosis and inflammation, such as TNF- α , NF- κ B, and various others, as well as reactive oxygen species, which can also come up with diabetic retinopathy. The current review also focuses on various therapies which can be used in the future, like SIRT1 signalling, resveratrol, retinal VEGF, etc., which can be used to suppress FOXO over activation and can prevent the progression of diabetic complications viz. diabetic retinopathy.

Keywords: Diabetes mellitus, diabetic retinopathy, FOXO, advanced glycation end-product (AGE), tumor necrosis factor (TNF)- α

Introduction

Diabetes mellitus (DM) is a persistent condition, characterized by high blood glucose levels that occur by virtue of insufficient insulin secretion or insulin resistance. Diabetes affected around 230 million people globally in 2010, with the number estimated to rise to 430 million by 2030. Diabetes has several serious health effects, including retinopathy, neuropathy, and nephropathy, associated with many diabetic complications. The results of several investigations showed that free radicals and upcoming oxidant stress-mediated hyperglycemia result in the occurrence of diabetes and its further progression into complicated health hazards [1]. Complications of diabetes are commonly identified in Type 1 or 2 diabetic patients,

although substantial morbidity and mortality occur. Diabetes is ordinarily divided into microvascular as well as macrovascular complications, with the prevalence of microvascular complications much greater than that of macrovascular ones. Neuropathy, nephropathy, and retinopathy are microvascular complications, while cardiovascular disease, stroke, and peripheral artery disorders are macrovascular complications [2].

Diabetic retinopathy (DR) pathogenesis is closely related to the interaction of retinal, neuronal, and glial cell disturbances. DR is one of the most encountered causes of diabetes and blindness, mainly in adults at work. A loss of pericyte coverage and acellular capillary formation is the first morphological predictor of DR.

Numerous lines of research have shown that angiopoietin-2 (Ang-2) plays a central part in initiating pericyte loss. Müller cells are the principal glial cells of the retina and are necessary to maintain the retinal microenvironment among retinal neuroglia, e.g., astrocytes and Müller cells [3]. Selective pericyte degeneration in retinal capillary vessels is an early histopathologic characteristic of DR. Pericytes of diabetic retinas are prone to apoptosis-related changes. Pericytes in the adult retina do not reproduce and degeneration tends to influence capillary permeability and macular edema [4]. The destruction of endothelial cells is found to contribute to the proliferation of focal capillary cells of the endothelial retinal cells, leading to a microaneurysm, or dysfunction of endothelial cells [5]. Pericyte apoptosis mechanisms are proposed to involve the development of advanced glycation end-product (AGE) and uveitis. Dosage and time-based apoptotic effects on pericytes are caused by AGE. Apoptosis of the endothelial retinal cells has also been demonstrated for tumor necrosis factor- α (TNF)- α with the proliferative type of DR in human retinas [6]. Surprisingly, anti-inflammatory medications protect diabetic retinopathy early events by suppressing TNF- and inhibiting TNF- in living beings, which reduces microvascular cell loss. While AGEs and inflammatory signals can both play a role in pericyte apoptosis, it's important to remember that these events are merely signalling signals [7]. Both TNF- α and AGE can encourage apoptosis by triggering the transcription fork-head box O1 (FOXO1) that changes the gene expression balance to apoptosis. The orthologues of the *Caenorhabditis elegans* fork-head factor DAF-16 are class O (FOXO) winged-helix transcription factors. Apoptosis through gene expression is modulated by the fork-head transcription factors FOXO1 (normally referred to as FKHR), FOXO3 (normally referred to as FKHR-L1) and FOXO4 (normally referred to as AFX) [8]. In general, FOXO upregulation affects the apoptotic expression of genes and stimulates roughly 25 pro-apoptotic genes which increase cellular fatality. FOXO1 is triggered and reduces acellular capillary formation as well as the formation of pericyte ghosts significantly in the retina of diabetic animals. The mitogen-activated protein (MAP) kinase pathway is possible via FOXO1, which could be triggered when responding to hyperglycemia. In the course of pathway p38, c-Jun nh2-terminal

kinase (JNK) and extracellular signal protein kinase, there are three major convergence points (ERK). The p38 and JNK generate pro-apoptotic signals in most cell types, whereas ERK typically mediates a survival signal (anti-apoptotic) [9]. Of course, FOXO1 was identified as high in diabetics, but FOXO1's effect on the genes of mRNA enhances glucose output thus contributing to diabetes hyperglycemia. DM can lead to increased FOXO1 activity and cells can lead to apoptosis, and thus FOXO1 can also play a major role in pericyte apoptosis. Even if diabetes could enhance FOXO1 activity and turn into cell death, it is obvious that FOXO1 can also contribute to pericyte cell death [10].

FOXO transcription factors: key regulator of cellular quality control

The FOX proteins are a superfamily of transcription factors that are evolutionary and play a major part in a broad range of biological mechanisms such as differentiation, proliferation, progression, apoptosis, metabolism, and migration. They are labelled as DNA binding domain (DBD), fork-head (FKH) or winged helix. Following their string homologies, far and wide the scope of DNA-binding, 50 human FOX proteins, divided into 19 sub-families (FOXA to FOXS), have been reported. Human FOX proteins are a wide family with significant versatility and complexity in regulation [11]. This family of proteins, the so-called FKH domain, is a conserved 100-residue binding DNA domain. The hepatocyte nuclear factor 3 (HNF-3) crystal structure study reveals that this domain consists of 3 major helices and two large wing-like loops. These proteins are also called transcription factors of the winged helix. The other distinguishing characteristic of FOXO proteins is the presence of strongly conserved phosphorylation sites for the kinase of survival Akt (a downstream PI3-kinase target (PI3K) signal) [12].

A subfamily has recently been established as having the same FOX factors as DAF-16 in the broader FOX family: Fork-head Box O (FOXO) factors. This subfamily has three mammalian proteins: FKHR (fork-head in rhabdomyosarcoma), FKHL-1 (FKHR-like one), and AFX (acute-lymphocytic-leukemia-1 fused gene from chromosome X). FKHR is also known as FOXO1, FKHL-1 is known as FOXO3a, and AFX is known

as FOXO4. All proteins of the FOXO family associate preferably with the core consensus area 5'-TTGTTTAC-3', even though the residues that flank the core contribute to the specificity of the DNA binding [13]. The protein in FOXO1 and FOXO3 (650 amino acids) is bigger than that in FOXO4 and FOXO6 (nearly 500 amino acids). In contrast to the other three family genes, the FOXO6 gene displays important structural variations. The four FOXO proteins have an overall homology in the sequence and four distinct functional motives, including the FKH domain, nuclear positioning, nuclear export, and transactivation [14]. The N-terminal domain can perceive two sensitive components of FOXO proteins: the insulin response factor (5'-(C/A) (A/C) AAA (C/T) AA) and the family member binding complement (5'-GTAA (T/C) AA) Daf-16 (namely, FOXO). While FOXO1 identifies both the insulin reaction as well as the Daf-16 binding factor, it is more closely related to the latter. The domain of transactivation positioned in the FOXO C-terminal domain blends with the FOXO-target genes' cis-regulatory locus [15]. Furthermore, to retain the FOXO proteins in the nucleus or cytosol, the nuclear localization and nuclear export sequences form part of the C-terminal DNA-attaching region of FOXOs. Nuclear FOXO proteins interact with DNA and partner proteins, primarily as transcription factors, to modulate various target gene transcriptions [16].

Description of FOXO proteins subtypes and their structures

Four members of the mammalian Fork-head transcription factors O-class (FOXO) include FOXO-1, FOXO-3, FOXO-4, and FOXO-6. FOXO1 and FOXO3 are revealed in virtually all the tissues, while FOXO4 is formerly evident in the musculature, kidneys, and colorectal, and in the nervous tissue and liver, FOXO6 is commonly seen. FOXOs stimulate or prevent downstream target genes transcriptionally, and thus play a significant role in proliferation, apoptosis, metabolism, and inflammation [17].

FOXOs synchronize a comprehensive variety of genes in the musculature linked with atrophy, along-with Fbxo32 Trim63 (MuRF1) and Atrogin1. If in muscle FOXO gets inactivated, it prevents debasing of muscles in response to hunger and nerve impingement. Deletion of the 3 FOXO muscle isoforms (FOXO1, FOXO3, and FOXO4) will rescue deep muscular debasing

from particular tissue deactivation of insulin resistance and IGF1R, revealing the crucial function of FOXOs in muscular protein deterioration and proteostasis [18]. The control of oxidative resistance to stress through the regulation of antioxidants and control of protein quality is the most crucial characteristic feature of FOXO transcription factors. Dysfunction of FOXO expression or stimulation leads to the focalization of the pathologic process of age-related diseases in the skeletal system, musculature, and central nervous system age-related diseases [19].

FOXO-1

FOXO1 is a module of the O-box subfamily FKH transcription factor. Different gene classes depending on the cell type and nature of the stimulus can be synchronized by FOXO1. Due to its pro-apoptotic effect through apoptotic gene regulation, FOXO1 has important tumor suppressor functions. It is also indispensable to safeguard hematopoietic stem cells from oxidative stress in the immune response [20]. It includes metabolism control, cell formation, oxidative stress-responsive response, homeostasis, embryonic stem cell pluripotency and death of cells. FOXO1 sets foot in and out of the nucleus respectively and is modulated positively by stress signals and negatively mastered by growth signals. The transcriptional activities of FOXO1 are governed by escalated nuclear entry or nuclear presence accompanied by their DNA binding capability [21].

FOXO-1 has been one of the leading insulin transcriptional regulators and is commonly revealed in all tissue types as well as in the insulin-like growth factor-1 (IGF-1) signalling pathway. In the fatty tissue of the viscera, nodes of lymphatic tissue, pleura, spleen tissue, and thymus, FOXO1 is particularly high in expression and organizes the development of many genes pertinent to adipocyte distinction, stress, and white-brown fat changeover [22]. FOXO1 regulates the cell cycle and cellular metabolism and the importance of FOXO1 has been manifested in many tissues, particularly in the liver and tissues with adipose [23]. In addition to phosphorylation, a diversity of post-translational changes have been found, like acetylation/deacetylation, to uplift the alterations in cell location, the levels of protein, the binding of DNA, and transcriptional action of FOXO1 and silent ma-

ting type information regulation (SIRT1), which are among the foremost agents governing the upstreaming of FOXO1 activities [24].

On chromosome 13, there seem to be three exons in humans of 111-kb genes. FOXO1 exon 1 and exon 2 encrypt the untranslated region of 5' (UTR) while exon 3 encrypts the 3'UTR with 655 amino acid (a.a.) prolonged proteins. FOXO-1 DNA-binding capacity, which covers 140 a.a. residues, comprises 4 helices (H1-H4) and 2 winged regions, is associated with the highly preserved FKH area (W1 and W2). The helix H3, which has a preserved N-X-X-RH-X-X-C/T sequence motif in the domain, is the main DNA identification component and attaches to the principal helix DNA groove. Within DNA, H3 is essential to recognize the 5'-CTAAA (T/C) AA-3' consensual sequence of the Daf-16 member binding element (DBE) in the target gene promoter. The FKH area of FOXO also links to the insulin-response element (IRE) with high affinity AAA (C/T) AA (C/A) (A/C). Although other members of the FOX family can recognize both the DBE and IRE motif, FOXO protein binds to a higher affinity DBE element. Each FOXO has a transactivation C-terminal, a nuclear location sequence (NLS), and a nuclear export sequence (NES) component besides the FKH domain. NES and NLS are situated between the transactive and FKH areas. For nuclear translocation of FOXO, NLS is required whereas NES is exported from the nucleus to the cytoplasm. The nuclear preclusion of FOXO-1 is mediated by the chaperon 14-3-3. The N-terminal component of the DNA linking framework and NLS are concerned with this interplay between 14-3-3 and FOXO-1 [25].

FOXO3

FOXO3 performs a vital function in the ultimate impact of increased lifespan on oxidative stress, damage to DNA, appetite, and caloric restraint. In particular, FOXO3 defends cells against the aggregation of reactive oxygen species (ROS) by controlling cell detoxifying and surviving genes. Two enzymes that execute an important role in ROS detoxification have been upregulated in reaction to the aggregation of ROS, which causes an increase in the expression of downstream transcription of superoxide dismutase-2 (SOD2) and catalase (CAT) [26]. The phosphoinositide 3 kinase (PI3K)/serine/

threonine specific (Akt) signalling pathway controls FOXO3. The functional, non-phosphorylated type FOXO3 is in the nucleus and controls gene transcription. In the PI3K/Akt pathway, phosphorylation of FOXO3 culminates in its elimination from the nucleus and the end of transcriptional activity [27]. FOXO3 contributes to immune system regulation. The immune system deteriorates over age and thus raises the risk of infection. FOXO3 contributes to the synthesis of human kidneys, lungs, as well as gut antimicrobial peptides. These battle microbial diseases in different organisms and also act as biomolecules of inborn immunity. Its anti-inflammatory activity includes inhibition of inflammatory cytokines production, including interleukin-2 (IL-2) and interleukin-6 (IL-6). The pleiotropic cytokine interleukin-6 is triggered in response to infection, and this controls the immune system in many forms, like antibodies, T cell activity, hematopoiesis, and inflammation [28]. FOXO3 deregulation is related to cancer development, increased Akt activity, or inactivation of PTEN by mediated regulation. FOXO3 is thus known as an anti-tumor protein [29]. FOXO3 is commonly known as the "Master" gene of human aging since it represents one or two genes that are strongly linked to aging and longing phenotypes and are replicated in numerous living organisms. Apo-E is a fatty acid transportation gene, which seems mainly to function in several age-related diseases, even though it is pleiotropic. The E4 allele is the major allele of risk. The functions of FOXO3 vary from tumor suppression to energy metabolism to old age-related illnesses such as heart diseases and cancer, or organic mechanisms such as oxidative damage, which have a broader impact on aging and the associated phenotypes. Cell eating, programmed cell death, breakdown of glucose and gluconeogenesis, cell proliferation/differentiation as well as stress resistance are important downstream objectives of FOXO3. Thus, it would be an important step to find the functional variant and its mechanism to understand human aging [30].

FOXO proteins have been expressed across several body tissues but are tissue-specific in terms of expression, function, and objectives. In the heart, brain, spleen, and renal and skeletal muscles, FOXO3 mRNA has been enriched to a certain extent. FOXO3 acts as the primary regulator for protein synthesis and breakdown

in muscles and is the key player in skeletal muscle protein turnover and a pivotal effector for PI3K/Akt signalling [31]. Akt phosphorylates FOXO3 in anabolic conditions and eliminates its transcriptional action. FOXO3 inhibitors, consecutively, lower the expression of ubiquitin-proteasome system enriched muscles, atrogen-1 (FBXO32) and RING 1 muscle finger (MURF1) [17], which encourage degradation of muscle proteins. Moreover, FOXO proteins can play their part in a negative feedback loop after Akt stimulation, which inhibits Akt's cell homeostatic stability. FOXO orthologues inhibit the mechanical target activities of rapamycin complex 1 (mTORC1) in non-mammalian cells that drive muscle protein synthesis downstream of Akt [32]. FOXO proteins decrease mTORC1 activity in mammalian tissue and activate Akt. Therefore, in the context of changing metabolic conditions, FOXO proteins can play an intricate role in balancing the activities of Akt and mTORC1. Catabolism caused by disease is also characterized by increased levels of expression of FOXO3 [33].

FOXO3a is around 71 kDa and is preserved throughout various species in structure. FOXO3a contains 5 domains: a helix-turn-helix DNA FKH, 2 NLS, a NEC and the C-terminal transactivation domain (TAD) [34]. Many of these regions are highly preserved by FOXO family members. The main responsibility for direct interaction between FOXO3a and DNA is the high-conservation FKH domain that also mediates their interactions with estrogen receptor α (ER α) and p53. The translocation of FOXO3a to the nucleus from the cytoplasm necessitates the NLS domain and likewise negotiates the release of FOXO3a. For trans-activating FOXO3a target genes, the C-terminal TAD domain is essential [35].

FOXO4

Initially, FOXO4 was encapsulated as a mixed-lineage leukemia (MLL) zinc-finger transcription factor of t (X; 11) chromosome translocation in acute leukemia, also known as the chromosome X (AF X) acute leukemia fusion gene. It was found that mammalian FOXO4 was expressed in different tissues, such as the nervous system, renal, pleura, epidermis, prostate gland, and musculature. Of these, the muscles of the skeleton are regarded as the most expressive place [36].

Like other FOXOs, it has just been shown that FOXO4 is a critical transcription factor in regulating a series of cellular processes. The expression of FOXO4 is administered closely by the non-coding RNAs, especially micro-RNAs, which depend on the modifying phosphorylation of their subcellular location, which is important to its performance [37]. FOXO4 is also widely identified by regulation of its antioxidant stress (AOS) targeting genes, cell division stoppage and programmed cell death as the key tumor suppressor. FOXO4 dysregulation has thus been thought to result in a large range of tumours. More findings have indeed demonstrated that anti-tumor medicines target FOXO4 activity in a clinic and include FOXO4 as a prognostic indicator of cancers [38].

FOXO4 is part of a subgroup called FOX 'O', which forms a crucial part of a sustained signalling pathway, linking growth and stress stimuli with the control of transcription. The fork-headed fold of the H1-H3 is made of three helices (H1-H3), the fourth shorter, the 310-H4 between H2 and H3, and the third, the non-parallel twisted plate with three sides of the S2 and S3 strands with residues of the leu118 and Thr119 strands acting as the 3rd strand of FOXO4-DBD (sequence 82-183). FOXO-DBD connects to the DNA duplex exactly in the same way as in other FOX-DBD-DNA frameworks, the H3 helix being mounted at a slope approximately perpendicular to the DNA axis [39]. The flexible wing of W1 between S2 and S3 and the turning zone between H4 and H3 are part of the DNA-contacting FOXO4-DBD and the N-terminal loop before the first helix H1. All of these areas have the greatest pattern of variability in the FOX class compared to the remaining FKH DBD, which may be part of the DNA-associating modulation. DNA produces pseudo-continuous helices through symmetry-related DNA molecular interactions [40]. The structure analysis revealed DNA small differences between the canonical B-form DNA. The mean turn of the helical is 32.7 with an average increase of base pair 3.1A. In the core-sequence region, the DNA molecule is geared towards FOXO4-DBD, with the main groove somewhat wider. The mean helical torque is 32.7, and the average increase is 3.1 per base-pair. The DNA molecule is twisted towards FOXO4-DBD around 13, with the main groove in the core sequential area being slightly wider. Such differences are analogous to those found in other structures of

FOX-DBD-DNA. The FOXO4-DBD-DNA complicated structure also showed a metal targeting location at the helix H3 C-terminal. This metal ion probably serves as a function to stabilize the twist from helix H3 to S2 strand through neutral neutralization of the helix dipole and water-mediated contact with the DNA backbone faction [41].

FOXO4 encodes a 505 amino acid protein and preserves its structure between various organisms. It consists of 4 sites including FHD, NLS, NES, and C-TAD as other transcription factors of FOXO. The FHD preserved in FOXO4 binds the kernel TTGTTTAC to the core common recognition motive and this is prohibited by insulin and insulin-like growth factor-1. For FOXO4 translocation to the nucleus, the NLS domain is required for communication with nuclear transfer engines, like chromosomal maintenance 1 (CRM1) that are bound to the NES. There is significant evidence, noted in various literatures. Moreover, the nuclear exclusion of FOXO4 from PKB-mediated phosphorylation is inactivated by the NLS [42]. FHD mainly acts as a significant connection between FOXO4 as well as DNA that can commence its attachment with certain proteins, like p53. The TAD in the FOXO3 C-terminal, however, also links to the p53 DBD. Since the TAD can maintain a balance between the FOXOs, the TAD in FOXO4 may often engage with multiple co-regulators, along with p53 [43].

FOXO6

FOXO6, a family member identified recently, its mRNA is exclusively revealed in the CNS in mammals, while FOXO1 and FOXO3 are relatively all-around expressed. The hippocampus is the main area for learning and memory and carries special FOXO6 mRNA. Like other members of the FOXO family, insulin/IGF signalling in cells negatively regulates FOXO6 [44]. FOXO6 phosphorylation in reaction to insulin/IGF signalling lowers the FOXO6-regulated transcription but does not influence the whereabouts of FOXO6 in the nucleus. These observations combine to raise the potential for FOXO6 to play a vital role in the hippocampus when the levels of insulin and IGF are stubby. Very few studies have questioned the method of activity of FOXO nervous system transcription factors [45]. The development of the FOXO family calls for neuronal polarity and FOXO6 ectopic expression can

rescue neuronal polarity defects because the FOXO family is inadequate. In adults, FOXO family members play a major part in the self-renewal as well as the destiny of neural stem cells. The erasing of FOXO1 or FOXO3 at organism levels reduces anxiety and depression. FOXO transcription factors have interestingly been detected as an insulin and IGF pathway that affects learning and memory in physiological or pathologic situations, including Alzheimer's disease. But the value and mode of action for cognitive behaviour of hippocampal enriched FOXO6 are unknown [46].

Three phosphorylation rationales were reported for FOXO1, FOXO3, and FOXO4. The first motif for PKB phosphorylation is located just below the start-codon, the 2nd one in the FKH region and the 3rd in an area just below the heading region. FOXO6 also shows 1st and 2nd areas with a motif of PKB phosphorylation. Strictly speaking, FOXO6 lacks the 3rd region that contains the PKB catalytic phosphorylation site [47]. In addition to the PKB phosphorylation region, it contains a further 3 serine residues that are accessible in the FOXO group's other members. The substrates for PKB in FOXO1, Ser-319, Ser-322, and Ser-325 are phosphorylated by CK1 and Ser-329 by DYRK1A. Even though FOXO6 is a high standard like FOXO3 and FOXO1, the preserved PKB site along with a residual stretch of the serine is not preserved [48]. It should be worth noting that in a region that is not similar to other FOXO and Daf16 proteins, the third motif of Arg-Xaa-Arg-Xaa-Xaa-Thr is located in the distant FOXO6 C-terminus. Moreover, in the domain, there are not any CK1 or DYRK1A motives. It is therefore not clear that the threonine residue is an unrefined substrate for PKB in this region [49].

FOXO and diabetic retinopathy

Diabetes mellitus is a metabolic disorganization that is competent at influencing all tissues, organs, and body mechanisms. Extended hyperglycemia and insulin deficiency result in several health problems like DR and also lead to delayed cure of all health problems. The development of diabetic complications is derived from increased oxidative stress and ROS. Many pieces of evidence suggest that the deletion or inactivation of FOXO protein may encourage cytoprotection and can show enhancement in insulin secretion during diabetes [50]. The

hampered FOXO1 expression and activity can be associated with diabetic complications. The liver is one of the most former as well as most encountered sites for the regulation of insulin response. Expression of FOXO1 in the liver comes with an idiosyncratic mechanism for the exaggerated production of glucose and increased fatty acid amalgamation and production [51]. FOXO1 often requires beta-cell distinction and pancreatic rejuvenation. FOXOs strongly induce atherosclerosis in endothelial cells, which can put an end to the generation of nitric oxide and augment the response to inflammation. The lower insulin production stimulates glucose-6-phosphatase (G6pase) activation and phosphoenolpyruvate (PEPC K) activation under rapid conditions, resulting in gluconeogenesis [52]. This metabolic reaction is largely dependent on AKT-FOXO1 crosstalk. FOXO1 takes part as a foremost character in arbitrating a bit of the sequels to insulin by synchronizing target gene elucidation [53]. FOXO1 consists of multiple profoundly conserved AKT phosphorylation spots (Thr-24, Ser-253, and Ser316), and its pursuits are modulated by AKT-mediated phosphorylation at these plots. Phosphorylated FOXO1 is eliminated from the nucleus by lowering its transcriptional activity, which is favourable for the expenditure along with the use of glucose by cells [54]. Yet, FOXO1 also enhances the expression of PEPCK and G6PC, the gene encoding G6Pase, by attaching to insulin-response components in the promoters of these genes [55].

FOXO1 activity is regulated by various processes besides phosphorylation caused by FOXO1 induction of AKT, like equilibrium between acetylation and deacetylation and SIRT1 deacetylation. Increased free radicals activate FOXO1 during physiological stress and mitigate the nuclear exemption impact of AKT [56]. This, therefore, promotes the nuclear translocation of FOXO1 intended genes (G6Pase & PEPCK) and their expression. SIRT1 regulates nuclear shuttles along with FKH transcription initiation regulators. SIRT1 either governs favourable or unfavourable activities of FOXO1 depending upon the target gene or cell type [57]. DR is caused by DM causing cell death in pericytes and microvascular endothelial cells, which increases the activation and expression of FOXO1. Experiments with diabetic mice in vivo have led to higher grades of FOXO1 mRNA and

nuclear transfer. In monitoring experiments, TNF- α mediates FOXO1, with FOXO1 exclusion via SiRNA reducing the threat of ocular microvascular endothelial cell injury [58]. In the generation of pro-inflammatory mediators, hyperglycemia-induced FOXO is important for prolonging the inflammatory phase. FOXO1 enhances mRNA values of BCL2 and CASP3, which promote cell death in laboratory diabetic experiments, according to mRNA sampling. FOXO1 also induces CCL2 and CCL5 expression under hyperglycaemic conditions, which induces vascular endothelium cells FOXO1 [59]. It also increases ITGA5 and ITGAV-M RNA expression, which controls angiogenesis. With DR, TNF- α and AGEs activate the FOXO1 transcription factor in-vitro and induce pericyte apoptosis [60] (**Figure 1**).

FOXO and oxidative stress in diabetic retinopathy

“Oxidative stress” is collectively recounted as a troublesome condition in the steadiness of antioxidants and pro-oxidants in favour of the latter due to multiple constituents such as aging, actions of drugs, and noxious insults to the tissue. Commonly, it happens due to superfluity formation, and/or insufficient elimination of highly reactive molecules such as reactive nitrogen species (RNS) and ROS [61]. The free radicals and the reactive oxygen metabolite with an unpaired electron (ROS) may cause potential harm to lipids, proteins, and DNA as they are powerful oxidizing agents. Small amounts of ROS are usually produced during the metabolic reaction by the mitochondrial respiratory chain but are appropriate and inevitable by-products of the breathing chain [62]. It is quite well-known that cells can intentionally generate ROS, and that in cellular processes, such as progression in the cell cycle, intracellular and extracellular signal pathway control, and inflammation, ROS is an important factor. Since high ROS levels can be damaging, cells have a wide range of antioxidant systems dedicated to ROS neutralization and keeping the balance between pro-oxidants and antioxidants [63]. Two types of antioxidants can be distinguished: enzymatic and non-enzymatic (or chemical). Proteins like SOD, glutathione peroxidase, and CAT are enzymatic antioxidants and chemicals are fungal molecules (scavengers) like vitamin C, vitamin D, and the

FOXO in diabetic retinopathy

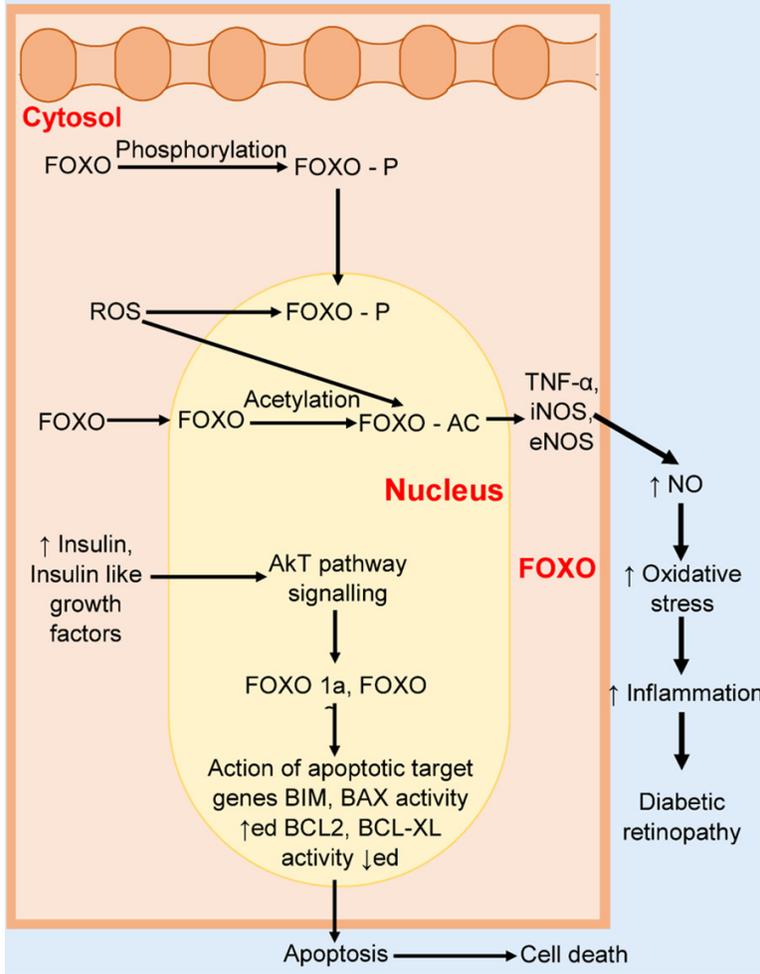


Figure 1. Schematic presentation of FOXO and ROS leading to cell death.

antioxidant glutathione (GSH). Pro-oxidants are induced nitric oxide synthase (iNOS) enzymes such as NADPH oxidase and cyclooxygenase 2 (COX-2). The resulting equilibrium determines the so-called oxidative stress whenever a close balance is disturbed between pro-oxidants and antioxidants [64].

Steadiness in linkage within the cost of free radical generation and eradication is very decisive in the headway of complication from diabetes. Literature has shown a raised level of ROS generation in diabetes as well as the initiation of diabetes in accordance with oxidative stress [65]. The real cause of oxidative stress in diabetes measures auto-oxidizing glucose or shifting redox equilibrium from averted glucose to the polyol pathway, which decreases the cell's overall reduction capacity. Moreover, reduced assemblage in tissue of antioxidants, such as

glutathione (GSH) or vitamin E, and disorganized happening of antioxidant safeguard enzymes, such as superoxide dismutase (SOD) and catalase (CAT), exaggerated vulnerability of the viscera to oxidative damage [66]. It is manifested that glucose-induced oxidative stress is a cardinal appliance in the focalization of pathogens of persistent complications of diabetes, and recent pieces of literature recommended that fibroblast units are a former target of oxidative vandalization [67]. Another look revealed multiple kinds of vessel units beget reactive oxygen species (ROS) under hyperglycemic conditions. Particular biochemical pathways get awake by oxidative stress, which is glucose-induced in the unit [68]. ROS emphasizes the generation of intracellular signals and awakes several pathways, such as protein kinase C (PKC), JNK, and p38 MAPK. These pathways lead to many functional changes, such as endothelial dysfunction mediated by the

activity of endothelial nitric oxide synthase (eNOS) and enhance the synthesis of superoxide [69]. Transcription factors are also activated, such as fork-head box O (FOXO) and nuclear factor kappa-B (NF-B), which can mediate the effects of ROS through regulation of gene transcription of inflammatory and antioxidant genes [70]. Oxidative stress also reflects DNA damage and together awakes DNA repair enzymes, transcription factors, and transcription co-activators in endothelial cells [71]. The functional consequence is increased transcription of multiple vasoactive factors and ECM proteins involved in chronic diabetic complications development and progression. The rundown comprises fibronectin (FN), collagen (COL), growth factor transformer (TGF), endothelin-1 (ET-1), endothelial vascular growth factor (VEGF), leptin, etc. Finally, ET-1 and VEGF are angiogenic factors shown to be highly expressed in the

eye of diabetes and mediators of PDR [72]. Glucose-induced oxidative stress consequently affects cellular signalling, tends to reflect alterations in the microenvironment and transcription, and causes chronic diabetic complications to develop over time [73].

Apoptosis

The capillaries of the retina are made up of pericytes and cells of endothelia. A mono sheet of luminous endothelial cells is surrounded by pericytes and their processes. Pericytes can participate in managing microvascular tone and contractility of the retina in this unique location [74]. Studies of endothelial cell and pericyte co-culture have shown that pericytes have both antagonistic actions on apoptosis and proliferation on cells of the endothelium. Pericyte death is an earlier episode in the development and progression of DR, with pericyte loss due to impairment of the non-cellular capillaries and enhanced vascular leakage. Non-cellular capillaries, in turn, lead to poorer development of DR pathogenesis, retinal tissue hypoperfusion, and oxygen deprivation [75]. Pericyte apoptotic mechanisms involve quick variations in glucose levels in the blood, hyperglycemia increased expression of Bax, AGE, and production of TNF- α [76].

FOXO1 is an important pericyte apoptosis downstream regulator of various apoptotic signals. Previously, the activation of FOXO1 was shown to cause pro-apoptotic genes to be induced globally and the intracellular balance to tilt apoptotically. In-vitro testing showed high FOXO1 DNA-binding action in reaction to TNF- α and AGE stimulus in retinal pericytes [77]. FOXO1 is known mainly for its insulin control effects on the production of glucose and sensitivity to insulin. Therefore, in addition to other effects, the increase in FOXO1 can lead to significant action in the pathophysiology of DR by pericyte apoptosis [78]. Several findings indicate that FOXO1 improves the binding activity and nuclear translocation of the microvascular cells of the retinas of type 1 and type 2 diabetic rats, and this siRNA knockdown of FOXO1 reduces acellular and pericytes ghost formation. Activation of NF- κ B is intricately linked to ocular diabetes complications in diabetic retinas pericyte and is recommended as another pathway used for retinal pericyte initia-

tion of cell death. NF- κ B inhibition increases apoptosis directly in pericytes in vitro, indicating that NF- κ B adds to the ability of cells to survive. NF- κ B can, however, even have indirect impacts, especially in a complicated multiple-cell atmosphere when its direct anti-apoptotic consequences can be overcome by the inflammatory effects [79]. The comparative influence of NF- κ B and FOXO1 activity depends on the apoptotic balance within cells. TNF α induces FOXO1, which stimulates pro-apoptotic genes before apoptosis. Of course, TNF- α also triggers the survival factor NF- κ B. This suggests that TNF- α induces apoptosis, employing a system consistent with the requirement for FOXO1 activation, which should endure the overall impact of the NF- κ B controlled "cell survival" genome [80]. The effect of TNF α was augmented by some other factors which usually get raised in diabetics, high glucose, and AGEs in a FOXO1 regulated mode. TNF α reduces the multiplicative capability of endothelial cells to react to growth factors. The latter is facilitated by FOXO1 and is driven by additional factors, such as high blood glucose and AGEs, which increase in diabetes [81]. Previous literature has demonstrated that the in vitro proliferative capacity of endothelial progenitor cells in type 2 diabetic patients has been reduced, according to our in vivo data [82].

A higher TNF α level can also influence endothelial cell figures by boosting cell death. TNF α inhibition saved elevated amounts of cell death in endothelial cells in vivo, and endothelial cell death in vitro was triggered by TNF α . Thus, it is shown that FOXO1 modulates the influence of TNF α , high glucose, and AGE on microvascular endothelial cells. All three significantly decrease the proliferation of endothelium and increase apoptosis. FOXO1 was required to improve the manifestation of p21, reduce cell growth and increase caspase-3, which is a weighty apoptosis-effector for each such effect. TNF α , as well as FOXO1, were also seen to play a limited role in the apoptosis of DR endothelial microvascular cells [83]. This seems to be an important pattern in pericytes in TNF- α -induced apoptosis because the TNF- α -induced cell death and FOXO1 siRNA suppressed TNF- α -stimulated cell death is enhanced by NF- κ B antagonists. Akt is also known to block FOXO transcription factors, including FOXO1, functional activity. It has been proved that Akt inactivation may con-

tribute to apoptosis in photoreceptor cells during retinal degeneration, whereas inhibition of Akt further enhanced apoptosis induced by TNF- α , showing that Akt functions as a retinal pericyte survival factor [84]. The inhibition of JNK and P38 MAP kinases significantly decreased TNF- α ability to activate FOXO1. The pericyte apoptosis induced by TNF- α was subject to these signalling pathways. JNK and p38 may therefore be pro-apoptotic, including FOXO1 activation. AGEs are recognized to increase cell functions, along with cell death, via RAGE signals and NF- κ B signals. In retinal endothelial cells, NF- κ B activation and apoptosis are stimulated by advanced glycations and DR increases activation of NF- κ B, linked to the in vivo RAGE signal [85]. While there is well-known evidence that NF- κ B is in numerous types of cells straight forwardly anti-apoptotic, NF- κ B can indirectly support apoptosis through the induction of apoptotic factors. AGEs can encourage the direct pro-apoptotic initiation of the FOXO1 transcription factors. Convergence with TNF- α stimulation on or before the MAP kinase route can suggest findings that JNK and p38 inhibitors decrease FOXO1 AGE activation. The study described here indicates that AGEs and TNF- α can add through FOXO1 mediated pericyte cell death to DR [86].

Mitochondria, ROS and FOXO

Electrical transport in mitochondria is an implementation via which diabetes can elevate oxidative stress. It was first found that high intracellular levels of glucose raise the number of electrons in mitochondria during oxidative breathing via the electron transport chain [87]. This may end up in the discharge of electrons to O₂, which leads to the development of O₂ and the production of different ROS in mitochondria. In addition, diabetes switching effects hamper redox balance or stability and vary in redox-sensitive proteins like C-epsilon, which may lead to the intensification of ROS production in mitochondria [67]. AGEs are produced under hyperglycemic conditions which stimulate NADPH oxidase, leading to ROS generation. An elevated amount of Wnt signal stimulates mitochondrial biosynthesis, which can boost mitochondrial ROS levels and contribute to greater oxidative damage in mitochondria [88]. Several modalities consider the increased ROS level in mitochondria to be troublesome.

One is that mitochondrial elements like DNA, membrane proteins, as well as lipids are vandalized by ROS. ROS can, however, promote the opening of the mitochondrial permeability transition pore (MPTP). If such a pore is managed to open, mitochondrial proteins like cytochrome c, which induce apoptosis, are discharged from the mitochondria. In the mitochondrial respiratory chain, ROS was proposed for NF- κ B stimulation by TNF- α and IL-1 as secondary messengers [89]. ROS can influence the activity of insulin signaling. In the conditions of oxidative stress, a reduction in insulin signaling is commonly seen, which may result in resistance to insulin. This can transpire via various processes. ROS initiates serine phosphorylation and reduces tyrosine phosphorylation of the insulin recipient substrate in one scenario, thus hampering the signals of insulin [90]. Similarly, the effects of angiotensin II inhibition of insulin signaling have been partly mediated by ROS. Methylglyoxal, a pharmacologically active AGE forerunner that was developed under hyperglycemic conditions, stops insulin receptor substrate phosphorylation and wakes up the PI3K/PKB pathway [91]. Insulin signals FOXO1, which is combined through Akt with insulin receptor substrates-1 and -2. The trait characteristic of insulin resistance is the formation of glucose levels in an increased manner, which ends up in hyperglycemia, an increased level of blood sugar. The production of glucose through the expression of genes that support gluconeogenesis in the liver is regulated by FOXO1. Therefore, there is a way of increasing insulin resilience by activating FOXO1, increasing gene regulation that promotes glucose production and therefore, also increasing serum glucose levels. The halt of the insulin-AKT-FOXO1 equilibrium also stresses mitochondria [92]. Triggered FOXO1 encourages heme oxygenase-1 (HMOX1), which splits heme and halts the mitochondrial electron transport chain. There is therefore increased expression of heme oxygenase-1 when FOXO1's activity is enhanced by insulin resistance. Greater hemoxygenase-1 level activity interfering with mitochondria has a negative impact on oxidative respiration and on the production of ATP. In addition, improved FOXO1 stimulation influences mitochondrial fusion and fission expression, thus impacting mitochondrial biogenesis. There are sparse mitochondria and an aberrant mitochondrial morphological characteristic in an atmosphere

of insulin resistance that is overturned when FOXO1 is deactivated [93].

Role of FOXO in inflammation in diabetic retinopathy

The inflammatory response incorporates a range of structural and cellular mediating variables and is not a particular response to stress or injury. However, if attacking microorganisms are involved, the pattern is recognized for receptors such as Toll-like receptors and AGE. Molecular patterns associated with pathogens (PAMP) are the molecules that bind to these receptors [94]. The transcription factor is de-inhibition NF- κ B, which translates into the nucleus to activate pro-inflammatory mediators. Acute-phase proteins, including chemokines in the case of tissue stress alone (IL-6, TNF- α , IL-1 β , and monocyte chemoattractant protein-1 (MCP1)), can inhibit the transcription factor. These pro-inflammatory chemicals perform an important function in enrolment as well as stimulation of monocytes and leukocytes and consequent inflammatory response [95]. Inflammation ordinarily works via a synchronized agenda that encircles resolvins, lipoxins, and protectins. If not fixed in time and with severe consequences, the typical effects of inflammation are lost. The greater finding demonstrates that inflammation plays a major role in developing DR [96]. Various inflammatory cytokines and chemokines are raised in serum and ocular samples of patients with diabetes. A range of inflammatory cytokines including IL-1 β , IL-6, IL-8, TNF- α , and MCP-1 were shown to be high in the eye tissues of non-proliferative DR (NPDR) patients. In diabetic eyes with NPDR, one study found IL-8 and TNF- α to be even higher than inactive PDR [97].

Inflammation has long been regarded as an important risk factor in the development and growth of diabetic complications in diabetes. Hyperglycaemia-induced oxidative damage encourages inflammation by increasing injury of the endothelial cells, increased permeability, and increasing leakage of pro-inflammatory cytokines, along with TNF- α , interleukin-1 β (IL-1 β), and IL-6. This eventually reduces the sensitivity to insulin and leads to the complications of diabetes [98]. In the activation of pro-inflammatory cytokines, hyperglycaemic FOXO plays a key role. FOXO1 is directly connected to the promoter IL-1 β and tends to increase macrophage

expression. FOXO1 is driven by inflammatory cytokines and can be part of a forward amplification circuit. Increased generation of IL-1 β and TNF- α are involved in obesity and diabetes pathophysiology [99].

NF- κ B also stimulates inflammation during hyperglycemia and oxidative stress. The progression of diabetic complications, in conjunction with DR, has been implied by the bracing of the NF- κ B pathway and has elucidated that several proinflammatory cytokines, like TNF- α and IL-1 β , have been regulated [100]. Chronically high diabetes-related ROS levels can cause both NF- κ B and FOXO to boost inflammation as well as cell injury. NF- κ B is directly antiapoptotic in most cell types, whereas FOXO1 is directly proapoptotic. Thus, their comparative equilibrium will determine if the cell finally stays alive or undergoes apoptosis in inflammatory conditions when both NF- κ B and FOXO1 are activated [101] (**Figure 2**).

Role of FOXO In angiogenesis in diabetic retinopathy

Angiogenesis is an intricate occurrence that includes the growth of endothelial cells, lumen, and tubulogenesis and is supervised by the collaborative activity of various factors of transcription. Their interplay tends to lead to the differentiation of the endothelial cells and the development of arterial, venous, and lymphatic properties [102]. Factors for transcription FOXO is an important player in all of these episodes and takes part in embryonic as well as adult angiogenesis. Both in embryos and adult mice, the subtype FOXO governs the appropriate organization of the vascular system to control exaggerated endothelial development and to lead to cell death [103]. Although trials advocate that FOXO1 plays a crucial character in forming and maturing the emerging blood vessels, an important process under which angiopoietin 1 (Ang1) modifies the endothelial role was demonstrated in fully grown endothelial cells to inhibit FOXO1 action. FOXO transcription factors by regulation of angiopoietin 2 (Ang2) and eNOS govern angiogenesis and postnatal neovascularization [104]. The significant molecule in the signal transduction pathway for angiopoietin is Tie2. At first, the interplay between Tie2 and Tie2 phosphorylation was regarded as the only way to achieve the metabolic activity of Ang2. According to previ-

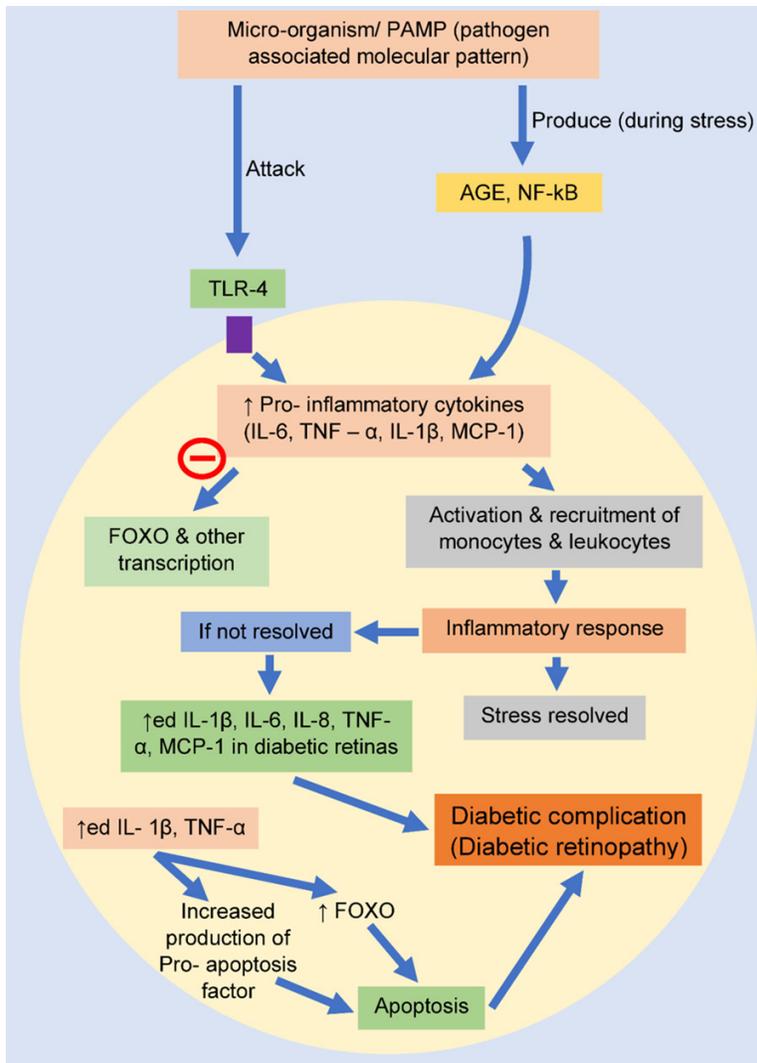


Figure 2. Pro-inflammatory cytokines responses in their exaggerated activity and their after effects.

ous literature, Tie2 is not often accountable for Ang2 mediated activities and interactions are recognized with certain other trans-membrane proteins [105].

The Tie2 receptor is represented in endothelial and hematologic stem cells, including all four angiopoietins. This tyrosine kinase receptor usually contains epidermal growth factor, immunoglobulin-like loops, and fibronectin type III repeats. It was first observed that Ang2 attaches, but doesn't really facilitate Tie2, and instead serves as an endothelial cell competing for Ang1 antagonist [106]. FOXO1 is manifested primarily in endothelial cells and is a deleterious angiogenesis modulator. Ang1, an effective activator of angiogenesis, inhibits the initiation

of FOXO by Akt. According to FOXO1 knock-down studies, phosphorylation in FOXO1 tends to lead to its inactivation and cytosolic location, thus suppressing Ang2 expression [107]. PI3K activation, preventing FOXO1 phosphorylation, triggering nuclear aggregation and therefore stimulation, contribute to a marked rise in ang2 mRNA amounts. The generated Ang2 autocrine was to activate Tie2 by phosphorylating Akt and, therefore, inhibit FOXO1 stimulation and FOXO1 influenced transcription and cell death to a level, but not fully. Phosphorylation of Tie2 was stimulated by Ang2 and chemotaxis and tubular development in endothelial murine brain cells [108]. Chemotaxis was facilitated by c-Fyn activation via PI3K, while tubular development was separated and influenced by c-Fyn activation via PI3K signaling. The orphan receptor, Tie1, has been found to take part in Ang1 and Ang2 angiogenesis control. Ang1, Ang2 ratifying on Tie2 results in Tie1-Tie2 interplay via β 1 integrin, while Tie1 cleavage reduces and loses Ang1 and Ang2 agonistic activities respectively by

inhibiting Tie2, Akt, and enhanced FOXO1 stimulation [109]. The stimulation of Tie2 is not only initiated by Ang1 or Ang2, but several growth attributes or cleaved Tie2 molecules can also trigger it. Ang2 inactivation of the lymph endothelial cells safeguards the phosphorylation, which causes button-like joints in the early lymph that deteriorate lymph absorption or vessel leaks due to disturbed attachment joints in the lymph collection [110].

The earliest and foremost changes in diabetes are endothelial derangement and are accountable for diabetic angiopathy. The silent information regulator1 communicates with the following biomolecules: FOXOs, P53, nuclear factor- μ , peroxisome, proliferator-activated receptor- μ

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Table 1. Types of FOXO proteins with their functions, whereabouts, and surrogate names

Protein	Alternative Name	Location	Function
FOXO1	Fork-head in rhabdomyosarcoma (FKHR)	Adipose tissue, lymph nodes, lungs, spleen tissue, tissue of thymus, pancreas, skeletal muscles	Oxidative stress resistance, metabolism, cellular differentiation, adipogenesis, glucose homeostasis, insulin sensitivity and aging
FOXO3	Fork-head in rhabdomyosarcoma like protein-1 (FKHRL1)	Cardiac, brain, spleen, kidney, skeletal muscle	Aging, Apoptosis, autophagy, oxidative stress resistance, inflammation
FOXO4	Acute leukemia fusion gene located in chromosome X (AFX)	Brain, kidney, lung, skin, prostate, muscle, in mucosal cell's nucleus of GIT	Cellular apoptosis
FOXO6	Fork-head box o 6	CNS mainly hippocampus	Memory Consolidation

coactivator-1 α , and myoblast determination protein, which are used to influence SIRT1 functions that are strongly defined in the vascular endothelium but are a part of the FOXO transcription factor family (FOXO1 is an endothelial modulator) [111]. Removal of FOXO1 in embryonic phases contributes to embryonic death due to serious vascular defects, indicating FOXO1's significant role in vascular growth. However, high blood sugar promotes nuclear translocation of FOXO1 in type 1 and type 2 diabetic rats and leads to cell death induction and deterioration of endothelial microvascular cells in the rats, which suggests that FOXO1 is an important deleterious transcriptional vessel formation controller [112] (**Table 1**).

Future therapeutic targets

SIRT1 signalling

Diabetes mellitus tends to affect several structures throughout the body, which comes out in both major disabilities and mortality. New approaches to targeting FOXO proteins can be developed for effective therapy of cellular metabolic rate disorder and DM medical comorbidities. However, in considering the clinical use of FOXO proteins, a lot of barriers must be resolved. Oxidative stress is a major mediator of DM cell damage. FOXO proteins increase cell viability, decrease oxidative stress and help with metabolic equilibrium. FOXO proteins may be activated in certain types of cells to mitigate apoptotic cell damage during oxidative stress. Certain situations might also necessitate initiation of the SIRT1 signalling pathway and suppression of FOXO protein expression, leading to cellular protection [113]. The discovery of the latest SIRT1 modulators led to lower cell death, inflammation, oxidative stress, and mitochondrial dysfunction. It also defends against DR. The underlying biomolecular procedure of DR is complicated, which can clarify effective treat-

ment objectives for the cure of DR through enhanced knowledge [114].

Melatonin

Retinal VEGF activity is associated with blood/retinal diabetic barrier breakdown, enhanced vascular permeability, and ischemic neovascularization, and is involved in background and propagative DR pathophysiology. Therapy maneuvers which eliminate excessive production of VEGF should be capable of preventing or diminishing DR's growth or progression. Studies are ongoing which suggest that cure with melatonin greatly reduces the upgraded expression of VEGF in diabetic retinal cells [115]. This data suggests that melatonin in DR has a promising therapeutic benefit that is partly due to its antioxidant activity. In numerous investigational settings, melatonin is an extremely efficient intracellular antioxidant and even has powerful anti-inflammatory effects. The prolonged, low-grade inflammatory response of the DR is culpable for most of these classic vascular lesions. In the eye and vitreous of diabetes, the concentrations of pro-inflammatory cytokines are raised. In the case of diabetic retinas, expressions of TNF- α , IL-1 β , and iNOS were significantly improved. Melatonin prevents stimulation considerably. The therapeutic efficacy of melatonin on uveitis and diabetic retinas was revealed to have similar findings [116].

Resveratrol

Many preclinical pieces of research suggest that resveratrol is involved with oxidative stress and inflammation-induced preventing and treating eye diseases. Both in vitro as well as in vivo data indicate that resveratrol has anti-apoptotic, anti-inflammatory, and anti-oxidative effects. It is realized that resveratrol acts primarily via oxidative stress amplification, and by

modifying certain metabolic processes such as inflammatory response, proliferation of cells, cell death, and angiogenesis [117]. There has been scientific proof that age-related ocular illnesses, such as DR, are initiated and progressive, leading to gradual visual impairment if left unresolved. Concerning such implications, medical applications are suggested for antioxidant and anti-inflammatory phytochemicals in age-related eye diseases. Thus, resveratrol is seen to delay the onset of debilitating vision problems among phytochemicals [118].

Conclusion

Diabetic retinopathy is the most familiar blinding complication of DM. FOXO transcription factors are key governors in cellular providence which play a significant role in influencing inflammation, oxidative stress, and apoptosis due to DR. ROS, AGEs induced by hyperglycemia, and upcoming oxidative stress are mainly necessitated in the etiology of DR. In normal situations as well as in normal individuals, FOXO plays a vital role in resistance to oxidative stress and has a protective role in various pathways of inflammation. While in DR there is an exaggerated triggering of FOXO due to which there is increased loss of endothelial cells, increased permeability, and increased generation of various pro-inflammatory factors and pro-apoptotic factors, which leads to increased damage to the retina and ultimately vision loss. Furthermore, there are numerous signaling pathways like JNK and MAPK that also coordinate FOXO in feedback to hyperglycemia-induced by diabetes. In the near future, research should be performed to learn more about numerous other pathways involved in the pathogenesis of DR, and studies should be conducted to see if therapeutic drugs targeting FOXO and other pathways involved in DR may be developed. This study also tells us in brief about therapies such as SIRT1 signaling, melatonin, and resveratrol which can be used to target FOXO transcription factors and prevent diabetic complications, mainly DR.

Acknowledgements

The authors express gratitude to Chitkara College of Pharmacy, Chitkara University, Punjab, India, for providing basic facilities for completion of the article. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

None.

Abbreviations

DR, diabetic retinopathy; DM, diabetes mellitus; Ang-2, angiotensin-2; AGE, advanced glycation end-product; FKH, fork head; HNF-3, hepatocyte nuclear factor 3 (HNF-3); FKHR, fork-head in rhabdomyosarcoma; FKHRL-1, FKHR-like one; AFX, acute-lymphocytic-leukemia-1 fused gene from chromosome X; *FBXO32*, F-box protein 32; *TRIM63*, tripartite motif containing 63; *MuRF1*, muscle RING-finger protein-1; ROS, reactive oxygen species; IGF-1, insulin-like growth-factor (IGF-1); SIRT1, silent mating type information regulation; UTR, untranslated region; DBE, Daf-16 member binding element; IRE, iron responsive element; NLS, nuclear location sequence; NES, nuclear export sequence; SOD2, superoxide dismutase 2; PI3K, phosphoinositide 3 kinase; PTEN, phosphatase and tensin homolog; MTORC1, mechanical target activity of rapamycin complex 1; TAD, transactivation domain; ER α , estrogen receptor α ; MLL, mixed-lineage leukaemia; AF X, acute leukaemia fusion gene chromosome X; AOS, antioxidant stress (AOS); TAD, transactivation domain; GSH, glutathione; iNOS, induced nitric oxide synthase; COX-2, cyclooxygenase 2; NF-kB, nuclear factor kappa-B; PDR, proliferative diabetic retinopathy; NPDR, non-proliferative DR; JNK, jun kinase; VEGF, vascular endothelial growth factor; ICAM-1, intercellular adhesion molecule 1; TNF- α , tumor necrosis factor-alpha; IL, interleukin; MCP-1, monocyte chemotactic protein 1; PAMP, patterns associated with pathogens; eNOS, endothelial nitric oxide synthase.

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