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Review Article

FOXO1: a pivotal pioneer factor in oral squamous cell carcinoma

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Abstract: The transcription factor FOXO1 regulates cell cycle progression, apoptosis and oxidative stress. Interestingly, numerous studies have implicated their positive role in tumor suppression, angiogenesis and metastasis in oral squamous cell carcinoma (OSCC). Distinct post-transcriptional and post-translational modifications actuate the physiological role of FOXO1 in OSCC. Here, we evaluate the role of FOXO1 proteins in OSCC, their fundamental structure and the major players involved in FOXO1 regulation and how they are Pharmacologically modulated in OSCC. Finally, their role in regulating epithelial-mesenchymal transition (EMT), autophagy, stress tolerance and stemness, which would significantly aid in novel potential oversight for future research and thus developing strategies to prevent or reverse OSCC.

Keywords: FOXO1, OSCC, microRNA, autophagy, stemness

Introduction

Oral squamous cell carcinoma is the sixth most prevalent cancer worldwide, which is characterized by the epithelial malignancies of the oral cavity and oropharynx [1]. Despite significant development in diagnosis and treatment modalities, the prognosis rate remains poor, largely due to the lack of early detection markers. To improve the overall patient survival rate, it is pivotal to better understand the nature of disease and identify specific biomarkers or signaling pathways that are essential in OSCC invasion and progression. Hence, an extensive investigation into the roles and functions of transcriptional factors are needed, to improve regiments in therapeutic front. These transcription factors are the most unswerving and promising targets for treating cancer; being the fact that only limited number of dysregulated transcription factors are there in cancer. However, an operational transcriptional factor to vary cancer progression remains indistinct. Toward the pursuit to better understand the mechanisms behind cancer, recent studies have illustrated the involvement of multiple transcription factors in OSCC tumorigenesis. Among these transcription factors FOXO received wider attention, as targeting FOXO could be a potential anti neoplastic therapeutic option.

FOXO1, referred to as Forkhead rhabdomyosarcoma transcription factor (FKHR), belongs to the larger family of Forkhead transcription factors [2, 3]. FOX genes obtained their name from Forkhead box, characterized by the presence of a distinct DNA binding region or the winged-helix domain, identified in Drosophila melanogaster [4]. It was first reported by Weigel and Jackle as a pivotal transcription factor [3] due to their inevitable role in an extensive range of biological processes, including regulation of cell proliferation, survival, DNA repair, cell cycle, apoptosis, metabolism and also immune regulation [5, 6]. Despite its crucial activity during various cellular functions, this protein has also been involved in myoblast, pre adipocyte and endothelial cell differentiation [7].

In mammals there are four genotypes, including FOXO1, FOXO3, FOXO4 and FOXO6 [5]. Of which, FOXO1 is said to have a crucial role in mammal's life as both the amino- and C-terminal regions of FOXO1 are imperative for linker his-

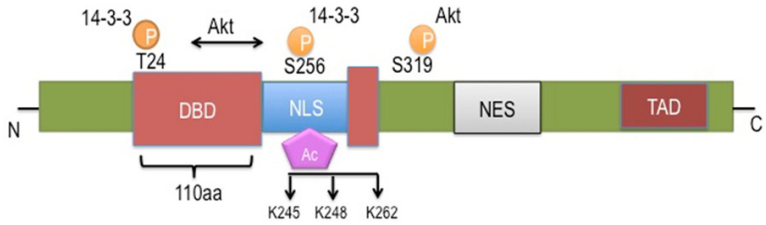

Figure 1. Schematic representation of FOXO1 with phosphorylation and acetylation sites.

tone remodeling in the normal tissues. Moreover, in nude mice complete disruption of FOXO1 contributed to embryonic lethality due to impaired angiogenesis whereas FOXO3 and FOXO4 ablated mice could continue to exist, substantiating FOXO1's essential role in various cellular activities [8]. Although FOXO proteins are tightly regulated to determine cell survival and cell death responsive to specific environmental conditions, inactivation of FOXO1 or increase p-FOXO1 expression often resulted in tumorigenesis [9].

The focus of the current review is to evaluate the pivotal role of FOXO1 in OSCC tumorigenesis. Therefore in the following section, we will provide a brief overview of the fundamental structure of FOXO and what are the major players involved in FOXO regulation in OSCC cells. Finally, their role in regulating cell cycle, epithelial-mesenchymal transition (EMT), autophagy, stress tolerance and stemness and how these current knowledge in FOXO would significantly aid in novel potential oversight for future research and thus developing novel therapeutic strategies to prevent or reverse OSCC.

Structure and function of FOXO1

X-ray crystallography and nuclear magnetic resonance analyses revealed a butterfly like structure for FOXO1 [10]. FOXO1s primary structure constitutes a highly evolutionarily conserved DNA Binding Domain (DBD) or winged helix domain, Nuclear localization signal (NLS), nuclear export domain (NES) and a C-terminal transactivation domain (TAD) [11]. The DBD comprises of 110 amino acids along with DNA binding domain (DBD) or winged helix domain, Nuclear localization signal (NLS), nuclear export signal forming a 3D structure. The DBD and specific regions within the N- and C-terminal domains facilitates FOXO1 binding to the DNA. The Akt phosphorylation and 14-3-3 binding site are spread across the DBD and NLS of FOXO1 [11]. Apart from these it has three acetylation sites, including K245, K248 and K262 by CBP/P300, three phosphorylation sites like T24, S256 and S319 by AKT and also two 14-3-3 binding sites, involving T24 and S256 [10] (**Figure 1**).

Regulation of FOXO in OSCC

MicroRNA and Long non-coding RNAs (lncRNAs) mediated post-transcriptional regulation of FOXO

FOXO expression is often found to be dysregulated in many cancers via multifarous mechanisms, such as gene mutation, chromosomal translocation or mutations in the upstream components of the regulatory pathways [12]. Recent evidence suggests that many microRNAs (miRNA) regulate the expression of FOXO proteins.

MicroRNAs are 22 nucleotide long small noncoding RNAs that regulates gene expression at post-transcriptional levels [13]. miRNAs facilitate post-transcriptional modification by binding to the 3' untranslated region (3'-UTR) of the target mRNA thereby repressing its translation [14, 15]. MicroRNAs, such as miR-196a have been reported to negatively regulate the expression of FOXO1 subsequently inhibiting apoptosis and triggering proliferation and migration in OSCC. In fact, miR-196a serves as an oncogene in OSCC. The down regulation of miR-196a resulted in decreased expression of p-P13k and p-Akt thereby upregulating FOXO1 expression [16]. Likewise, miR-155 promotes proliferation of OSCC by regulating BCL6 and cyclin D2 expression. Typically, miR-155 exerts its regulatory role by binding to the 3'UTR of the targets, such as FOXO3, SOCS1, claudin-1 and also BCL-6/cyclin D2 regulates cell cycle and proliferation by modulating pro-HB-EGF and FOXO, hence, an indirect trigger for FOXO [17]. In addition, miR-194 inhibited PI3K/AKT/FOXO3a signaling pathway through the direct suppression of acylglycerol kinase (AGK), triggering p21 expression and reducing cyclin D1 expression [18]. Notably, the upregulated exosomal miR-155 in oral cancer promoted migration, metastasis and cisplatin resistance

through FOXO3a regulation [19]. Thus targeting miR-155 along with conventional chemotherapy might aid in to overcome chemoresistance in OSCC. In addition, long non-coding RNAs Growth arrest specific 5 (GAS5), acts as a competing endogenous RNA (ceRNA) of miR-1297, inhibiting miR-1297 expression on propofol treatment. Upon propofol treatment FOXO1 directly binds to GAS5 promoter and facilitates its transcription. These elevated GAS5 binds to miR-1297 and abrogates its expression triggering apoptosis in OSCC cell [20]. Overall, one mechanism of FOXO regulation in OSCC is through miRNAs and lncRNAs, asserting its importance in regulating tumorigenesis and metastasis in OSCC. However further studies are warranted to establish after what precedent the miRNA and lncRNA regulates the expression of FOXO in OSCC.

Besides, post transcriptional regulation the cellular activity of FOXO1 largely relies on post-translational modifications, including phosphorylation, acetylation, ubiquitination, methylation and glycosylation [9]. These post-translational modifications are apparently modulated by two complementary mechanisms: (a) altered subcellular localization and (b) regulation of DNA-binding and its interaction with other DNA binding proteins [6]. These can have an abysmal effect on gene transcriptions regulated by FOXO.

Phosphorylation modification is a cutting edge process that determines sub-cellular localization and transcriptional activity of FOXO proteins. Phosphoinositide-3-kinase (PI3K)/Akt pathway has been reported to be up regulated in OSCC [21] and also Akt activation abrogates FOXO transcription mediated apoptosis in OSCC. Upon phosphorylation by PI3K/Akt or serine/threonine-protein kinase serum/glucocorticoid-regulated kinase 1 (SGK1), FOXO1 is pushed out of the nucleus to the cytoplasm [5, 22, 23], which is mediated by 14-3-3 [24]. 14-3-3 binding on to the phosphorylated FOXO1 attenuates its DNA-binding ability promoting FOXO1 nuclear export where these transcription factors are sequestered and are earmarked for degradation via ubiquitylation-mediated proteasome pathway [25]. The serine threonine kinase AKT is an important downstream target of phosphoinositide 3-kinase (PI3K) signaling [26] and has been reported that upon phosphorylation by Akt, FOXO1 are excluded from the nucleus and are ubiquitinated in the cytoplasm ensuing its degradation in the 26 S proteasome [27]. FOXO1 is also phosphorylated by ERK and p38 [28]. On direct phosphorylation on nine serine residues by ERK modulates FOXO1's co activator function of Est-1 on the fetal liver kinase (Flk-1) promoter. This implies that the phosphorylation status of FOXO1 by ERK and p38 determines its co activator activity for Est-1, which is associated with angiogenesis [28]. Apart from PI3K/Akt, ERK and P38, c-Jun N-terminal kinase (JNK) or macrophage-stimulating 1 (Mst1) can also phosphorylate FOXO1. This JNK mediated phosphorylation results in the import of FOXO1 from cytoplasm to the nucleus, thus negating the effect of PI3K/AKT phosphorylation [29]. Importantly, phosphorylation modification is a critical event in determining the transcriptional activity of FOXO1 proteins; it is also responsible for determining the sub cellular localization and stability of FOXO1. Even though many phosphorylation sites have been discovered the exact role in bringing about their co activator activity in OSCC remains an intriguing challenge.

Apart from phosphorylation, acetylation also steers the transcriptional activity of FOXO1. FOXO1 acetylation by p300/CBP promotes the transcriptional activity of FOXO1. However, acetylation is a reversible process [30] Deacetylases like sirtuins and HDACs can deacetylate FOXO1. Sirt1, an NAD-dependent class 111 HDAC binds to the coactivator-interacting LXXLL motif, aa 459-463, of the FOXO1 [31] bringing out a duplex effect on FOXO1 regulation: (1) Increases its ability to bring about cell cycle arrest (2) Also, impeding its ability to induce cell death [32]. This duplex regulation of FOXO1 by SIRT1 can be attributed to its retailored DNA binding ability. Apparently, Sirt1 modulates the DNA-binding activity of FOXO1 depending on the target gene promoter, which they bind to and also determines the sub cellular localization of FOXO1. It was found that SIRT1 over expression inhibited EMT through the regulation of epithelial marker E-cadherin as well as the mesenchymal markers N-cadherin and vimentin in OSCC cell lines. SIRT1 repressed migration and invasion via deacetylation of Smad4 and downregulating MMP-7 in OECM1 and HSC3 cell lines [33].

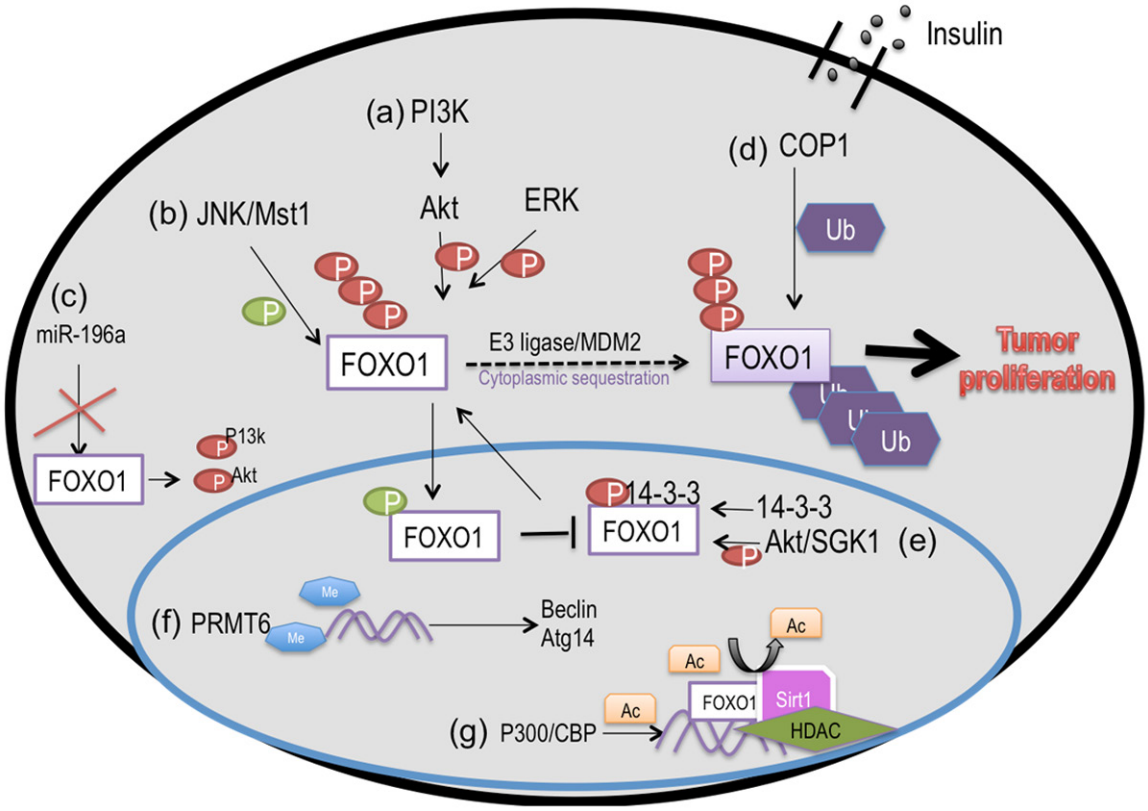

Figure 2. Regulation of FOXO1 in OSCC. (a) FOXO1 regulation by PI3K/Akt/ERK pathway. Insulin mediated FOXO1 degradation. (b) JNK/Mst1 mediated FOXO1 export from cytoplasm to nucleus. (c) miR-196a mediated FOXO1 regulation (d) Insulin signaling regulates FOXO1 via COP1 (e) Akt/SGK1 mediated FOXO1 nuclear exclusion. (f) Autophagy induction via FOXO1 regulation by PRMT6. (g) p300/CBP mediated FOXO1 transcription PMRT6 methylation of FOXO1 triggers the activation of beclin and Atg14 genes.

Similarly, ubiquitination is also essential for protein degradation. The addition of ubiquitin to a client protein will mark them for degradation through the proteasome or will alter their sub cellular localization. Ubiquitination is a three major step process characterized by activation, conjugation and ligation, which are executed by ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin ligase (E3) respectively [34]. Ubiquitin modification can be of either monoubiquitination or polyubiquitination. ERK, an upstream FOXO kinase, modulates FOXO1 degradation through E3 ubiquitin ligase, murine double minute 2 (MDM2) [30]. MDM2 can also promote FOXO1 degradation via AKT phosphorylation whereas insulin signaling also regulates FOXO1 via COP1, an E3 ubiquitin ligase. Insulin seems to enhance COP1 expression by polyubiquitination [30]. However, insulin that mediates FOXO phosphorylation in COP1-FOXO1 interaction largely remains to be identified in OSCC. **Figure 2** depicts the regulation of FOXO1 in OSCC.

Role of FOXO1 in OSCC

FOXO1 transcription factor is considered to be a tumor suppressor due to its inevitable role in the orchestration of gene expression that modulates cell cycle progression, apoptosis and oxidative stress resistance [35]. FOXO's can mediate both intrinsic and extrinsic apoptotic pathways through the activation of pro apoptotic genes such as Tumor necrosis factor (TNF) related apoptosis (Trail), FAS-ligand (FasL) that activate TNFR, FasL, Bim and BMF [36]. For example, FOXO on activation triggers apoptosis by initiating the transcription of tumor necrosis factor ligand 6 or FasL, ligand responsible for the Fas-dependent cell death pathway and also by up regulating pro-apoptotic Bcl-2 family member Bim [36]. On the other hand, FOXO proteins can initiate cell cycle arrest by upregulating p27kip1 to induce G1 arrest or GADD45 to induce G2 arrest eliminating the chance of further mutation [37]. Mechanistically, FOXO proteins can regulate the transcription of genes

involved in the generation of reactive oxygen species (ROS) by stimulating peroxidase catalase and manganese superoxide dismutase (MnSOD), which are responsible for the detoxification of reactive oxygen species [30, 38].

FOXO1 in stress tolerance

Oxidative stress produced by reactive oxygen species (ROS) is considered to be a hallmark for the development and progression of OSCC. Higher ROS levels in cancer cells stimulated oncogenic phenotypes such as proliferation, invasion and metastasis [39]. Though ROS are essential for the normal physiological functions, its overproduction has been detrimental to the cells. Normally, cells counter this detrimental effect of ROS by triggering enzymatic scavengers or modulating transcription factors, one among them is FOXO.

FOXO proteins are well known regulators of oxidative stress resistance that functions through the regulation of antioxidants. Within the FOXO proteins, FOXO1 is the main regulator of oxidative stress [40] and its abrogation altered redox balance in osteoblasts. Deletion of FOXO1 reduced mitochondrial superoxide dismutase 2 (SOD2) activity specifically upregulated Gadd45, a stress activated DNA repair gene and down regulated the pro-apoptotic gene FasL. FOXO1 deficient mice induced oxidative stress via cells proliferation stress affecting glutathione (GSH) accumulation, an intracellular antioxidant protein responsible for ROS neutralization, implying FOXO's inevitable role in regulating oxidative stress and curbing its detrimental effects [40]. Furthermore, under oxidative stress, FOXO1 detaches from SIRT2, a NAD+-dependent histone deacetylase and get acetylated to remain in the cytoplasm. This acetylated FOXO1 remains in cytoplasm and inhibits tumor growth in an autophagy dependent manner [41]. However, purview of developing therapies against ROS remains a major challenge since approaches that do not hamper the normal ROS needs to be achieved in this front. Proper understanding on interruption of ROS management machinery in OSCC may provide potential strategies to curb or inhibit tumorigenesis. Thus, the FOXO1-mediated ROS regulation might serve as a novel therapeutic intervention in the future for the treatment of OSCC. **Figure 3** details the role of FOXO1 in OSCC.

FOXO1 in autophagy

Autophagy is a unique self-degradative process characterized by the sequestrations of cytoplasmic constituents including damaged proteins and organelles, which are trafficked to the lysosome for degradation [41]. In this, the cytoplasmic bulk is first sequestered by a phagophore resulting in the formation of a double membrane structure called autophagosome. On fusion with the lysosome, the lysosomal enzymes degrade these autophagolysosomes [41]. Accumulated evidences have shown that in cancer cells regulation of autophagy may be an effective strategy to halt cancer growth and invasion, as it is revealed that most of the autophagy modulators are frequently dysregulated in almost all common malignancies. For instance the frequent allelic deletion of essential phagocytic gene beclin-1 is associated with 40-70% tumorigenesis of several human cancers [42]. Since the autophagy is either associated with cytoprotection or cell death the cord that links the autophagic and anti-neoplastic activity needs to be elucidated. Wherefore, more insights into the molecular aspects by which the autophagy is regulated could equip us to find new therapeutic interventions. Interestingly, the mammalian FOXO transcription factor has a multifaceted role in autophagy regulation. FOXO protein induces autophagy in genomic and non-genomic way. FOXO protein elicits autophagy by binding to the promoter region thereby transactivating the expression of genes that induces autophagy. A role for FOXO1 protein in autophagy is been established through an invitro study revealing that the knock down of oncogene, SRSF3 induced autophagy by increasing Beclin-1 expression via the upregulation of FOXO1 and p65 [43].

Studies have reported that in hepatocellular carcinoma HepG2 cells, chromatin factor high-mobility group A1 (HMGA1), an architectural transcriptional factor binds to the promoter region of endogenous FOXO1 gene thereupon increasing the FOXO1 mRNA and protein levels leading to the activation of genes involved in gluconeogenesis [44]. However, HMGA1 was reported to down regulate autophagy in human squamous carcinoma SCC-13 and HeLa cells. On knockdown of HMGA1, there was an increase in expression of autophagy-initiating kinase Ulk1 gene. Chromatin immunoprecipitation assay revealed that HMGA1 repressed

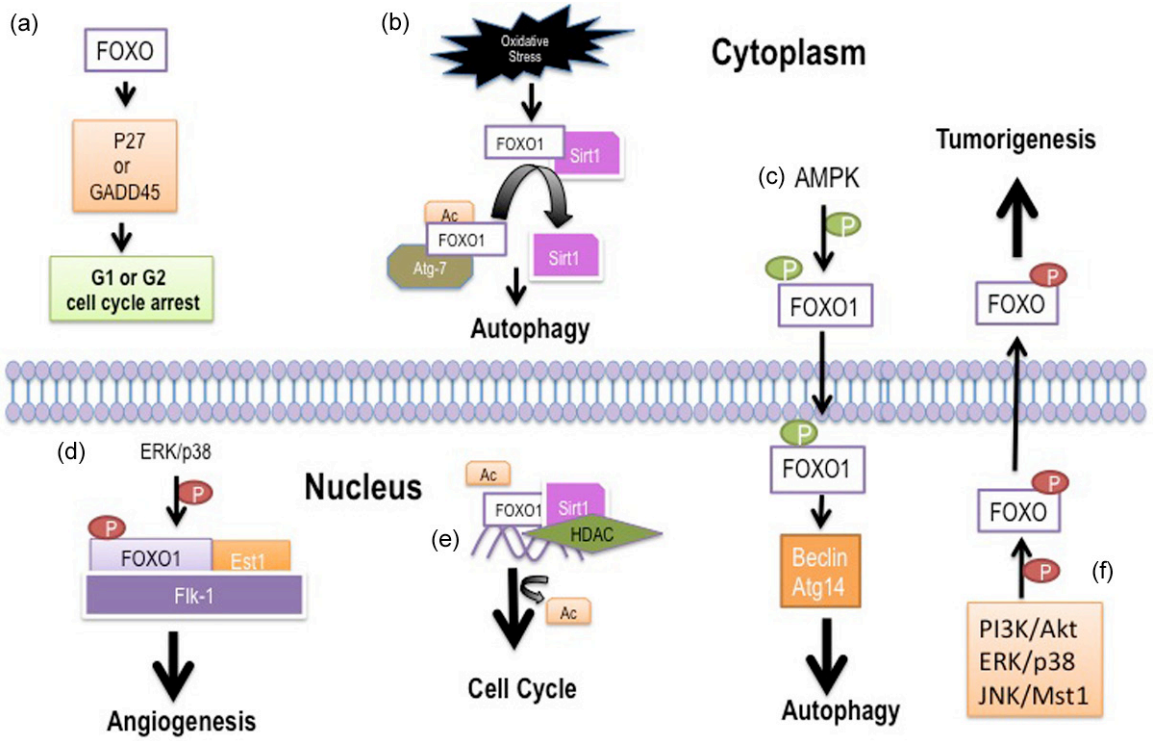

Figure 3. Role of FOXO1 in OSCC. (a) FOXO1 induces cell cycle arrest through p27 and GADD45 upregulation (b) Oxidative stress induced autophagy via FOXO1 acetylation FOXO1 in epithelial-mesenchymal transition. (c) AMPK induces autophagy via FOXO1 regulation by. (d) ERK/p38 phosphorylation of FOXO1 determines the co activator activity of Est-1. (e) SIRT-1 and HDAC deacetylates FOXO1 and increases its ability to induce cell cycle arrest. (f) PI3/Akt, ERK/p38, JNK/Mst1 induces tumorigenesis via FOXO1 phosphorylation.

Ulk1 by binding to its promoter [45]. Unlike HMGA1, Ulk1 gene is also a target gene for FOXO protein but their precise role in HMGA1 mediated autophagy through ULK1 repression remains elusive [46]. There could be other added mechanisms responsible for the regulation of FOXO-autophagy axis other than HMGA1 warranting more in depth studies.

FOXO proteins may also regulate autophagy flux through other mechanisms like post-translational modifications, chiefly by direct interaction with autophagy related proteins such as cytoplasmic Atg7, an E1 like enzyme, to initiate autophagy [41]. The cytosolic FOXO1 has been implicated to have a distinct function in autophagy induction. Here the FOXO proteins translocate to the nucleus from cytoplasm on phosphorylation by AMPK [47] and also by PRMT6-induced methylation triggering the activation of Beclin 1 and Atg14 genes. Consistent with this, studies have reported that FOXO1 methylation by PMRT1, protein arginine methyltransferase 1 at Arg 248 and Arg 250 inhibited Akt mediated phosphorylation [48]. Phosphorylation of FOXO1 by Akt, is known to induce FOXO1 cytoplasmic localization thus promoting FOXO1-Atg-7 interaction. Apparently PMRT1 repression triggered Akt mediated FOXO1 phosphorylation at Ser 253 resulting in the export of FOXO1 from nucleus to the cytoplasm where they are ubiquitinated for proteosomal degradation [48]. Wherefore further studies are called for to unravel the role of PMRT1-mediated FOXO1 activation in the regulation of autophagy. A number of studies evidenced that apart from phosphorylation and methylation; FOXO1 acetylation is also a perquisite to elicit autophagy. In human colon tumors, in response to oxidative stress or serum starvation FOXO1 protein gets dissociated from sirtuin-2 (SIRT-2), which is a NAD+-dependent histone deacetylatase and upregulates the acetylation of its substrate FOXO1. This acetylated FOXO1 binds to Atg7, an inevitable protein in the formation of autophagosome results in tumor suppression via autophagy [41]. It is also worth noting that SIRT-2 is downregulated in OSCC. There activa-

tion in OSCC triggered inhibition of migration and metastasis to the lungs [33]. Further understanding about the factors responsible for stabilization and how it's been operated would widen our scope of new therapeutic interventions. In parallel, a future investigation into the mechanism behind the cytoplasmic retention of FOXO1 and the process by which it circumvents proteosomal degradation is imperative for the treatment and prevention of OSCC These corroborated evidences imply that the FOXO-autophagy axis would be a cutting-edge break through in preventing OSCC.

FOXO1 in epithelial-mesenchymal transition

The epithelial-mesenchymal transition (EMT) is a bidirectional transition process in which polarized epithelial cells undergo various biochemical changes that will aid in to acquire mesenchymal cell phenotype. This mesenchymal -like properties confer them with enhanced migratory and invasive properties with an elevated resistance to apoptosis, a feature that is distinctive to malignant cells [49]. The role of EMT in cancer metastasis has been widely explored over 30 years. During EMT, cancer cells detach from the primary site of the tumor and are migrated and disseminated to distant sites promoting dismal metastasis. Since EMT is known to promote cancer metastasis, its regulation could serve as a promising anti cancer choice. So far a potential target to facilitate EMT inhibition or reversal remains elusive. Of the many molecules, FOXO1 have drawn much attention in recent years because of their anti cancer activity. They are known to exert anti cancer activity by modulating EMT- associated signaling pathways whereby inhibiting proliferation, invasion and metastasis in cancer cells. Preclinical data suggest that FOXO1 has the ability to influence the major EMT players i.e. upregulation of FOXO1 expression inhibits EMT and metastasis [1]. FOXO1 knock down in Cal-27 cells resulted in repression of E-cadherin and B-catenin while there was a surge in the expression of N-cadherin and vimentin both in mRNA and protein levels [1]. Further work on EZH2 abrogated Cal-27 and Tca8113 cells confirmed the role of FOXO1 in EMT. EZH2 knock down downregulated STAT3, a latent transcription factor that has been alarmed to disrupt the FOXO1 transcription, enhancing the FOXO1 expression. The enhanced FOXO1 expression elevated the expression of epithelial markers abating mesenchymal marker expression [50] confirming the clinical significance of STAT3-FOXO1 in OSCC invasion and metastasis. Though multiple factors are responsible for EMT in cancers, FOXO1 abrogation seems to be the major event in driving EMT. Therefore, development of future therapies targeting FOXO1 is of high significance.

FOXO1 in stemness

The pivotal role of FOX transcription factors in differentiation and development of various organs and its extended expression in multipotent progenitor cells underscores its ability to impart stem cell like properties in many cancer cells. Their role in embryonic development is yet to be studied in detail. FOXO1 proteins are found over expressed in mouse embryonic stem cells (mESCs) as well as human embryonic stem cells (hESCs). Upon differentiation their level seems to get reduced [51] intimating its importance in embryonic stem cells. In fact the protein-protein interaction studies have revealed FOXO1`s bindings to specific OCT4 and SOX2 regulatory regions up regulates OCT4 and SOX2 expression [51]. This was further confirmed through luciferase assay highlighting its lineage dependent specificity. In this respect, it's critical to understand the influence of FOXO proteins in maintaining the self-renewing capacity to design better therapeutic strategies. Genome-wide analysis of FOX proteins interactions with core regulators, including OCT4, SOX2 and Nanog would transmit more information regarding the involvement of various signaling pathways and their master regulators, which will equip us with a framework of factors involved in promoting pluripotency.

Implication for cancer therapy

Till date, many chemical compounds have gained considerable attention for their therapeutic potential in treating cancer. Among them many have elicited their therapeutic potential via the regulation of FOXO proteins. The role of FOXO in regulating various cellular processes reinforced their pharmacological importance in OSCC. For example, pitavastatin induced anti cancer activity through FOXO3a regulation [52]. It induced the nuclear translocation of FOXO3a through Akt-AMPK regulation triggering the expression of PUMA i.e through the induction of intrinsic pathway [52]. Similarly, rapamycin

combined with cisplastin-elicited a synergestic anti cancer activity via FOXO3a reactivation in OSCC Tca8113 cell lines [21]. Specifically, in Tca8113 rapamycin prompted FOXO3a stability via the down-regulation of Skp2 resulted in the feedback activation of Akt whereas cisplatin reactivated FOXO3a through the inhibition of Akt [21]. Few naturally derived compounds have also been shown to inhibit cancer progression through the activation of FOXO proteins. A natural precursor of resveratrol, polydatin inhibited Akt and STAT3 pathway inducing FOXO1 expression suppressed migration and invasion in hepatocellular carcinoma [53] Quercetin, (3,3',4',5,7-pentahydroxyflavone), a major dietary flavonoid induced its anti-cancer effect via FOXO1 mediated p21/FasL cell cycle arrest and apoptosis respectively in oral squamous cell carcinoma [54]. Isorhapontigenin, a resveratrol analogue inhibits STAT1 to activate FOXO1 limiting invasion in bladder cancer cells [55] A very recent study has verified FOXO1 as a key modulator for actein in its anti-OSCC effects. Actein treatment significantly reduced AKT phosphorylation and upregulated FOXO1 in OSCC cell lines. Further FOXO1 abrogation in OSCC reversed the actein-mediated apoptosis and cell cycle arrest substantiating its therapeutic importance [56]. Although the underlying mechanism of action for these compounds is known, these natural compounds are not yet into the clinics for treatment or prevention of cancer. Yet these studies strengthens that FOXO activity is crucial in the regulation of tumorigenesis and metastasis [35]. Anyhow, FOXO proteins are down regulated in many cancers and are key players in drug resistant cancer; targeting FOXO proteins would form an antecedent for treating OSCC. In essence, the tumor suppressor FOXO interpose as a key anti cancer agent in OSCC.

Future perspectives

The fundamental role of FOXO1 proteins in maintaining cellular homeostasis has been well characterized in recent studies. Its functional versatility in integrating various signaling pathways highlights their potential clinical relevance. Even though studies have addressed their role in drug resistance, little is known about their mechanistic action. More insight into the mechanistic role of FOXO1 proteins in the process of drug resistance would framework the molecular mechanism involved in it. Thereby discovering more specific and targeted therapeutic approaches catering the needs of patients who are resistant to the current treatment modalities.

Evidences suggest that plethora of functions attributed to FOXO1 proteins can be regulated through certain small-molecular compounds. [57]. In line with this, the identification of FOXO1 stimulating factors would aid in activating apoptosis in cancerous cells and also in preventing angiogenesis by inhibiting certain signature angiogenic markers like matrix metalloproteinase 9 (MMP9) [58]. Over the years substantial progress has been made in discovering compounds that can activate FOXO proteins and there have been promising efforts to develop potential drugs targeting FOXO proteins such as selinexor, a selective inhibitor of nuclear export (SINE) [59, 60], but its safety and tolerability still remains a concern. Harboring organic compounds will help to overcome the unpredictable adverse effects posed by these synthetic drugs. Notably natural compounds have gained much attention among researchers due to its affordability, effectiveness in treatment and also its high bioavailability. Despite this, the correlation between healthy diet and low cancer incidence prompted researchers to explore more into the chemo preventive effects of natural compounds [39]. To this end, identifying natural compounds that can trigger FOXO1 activity holds promising therapeutic strategy in OSCC. For instance many studies have pointed out the effectiveness of phytochemicals in treating and preventing OSCC but are not yet into the clinics. Hence further investigation into its effectiveness and safety in clinical use will lessen the OSCC tumor burden without eliciting significant off target effects.

Interestingly, the presence of highly conserved DNA binding domain remains a distinct advantage, targeting small molecules to these conserved regions of FOXO1 specifically activate or stimulate this particular transcription factor. Similarly, the intrinsically disordered regions (IDRs) also serve as a druggable target especially when they are folded and bound to their binding partners [61]. Since these IDRs display unique roles in transcription through liaising with distinct interaction partners [62] profiling

and targeting various FOXO1 interacting partners might serve beneficial to develop innovative therapeutic agents, particularly against 14-3-3 [63]. Together with, a better understanding about FOXO nuclear localization signal would unravel several other clinically relevant targets. Accordingly, targeting FOXO modifying enzymes such as HATs, HDACs, methyltransferases and ubiquitin ligases might help to modulate FOXO function along with additional targets. Additionally, a number of other factors influence the FOXO1 protein activities, including protein stability, post-translational modification, micro RNA level and change in chromatin landscape. A clear-cut understanding about FOXO1 regulating factors, its localization pattern and binding partners would pilot more rational approaches for FOXO1 based therapeutics in OSCC. Overall, crucial role of FOXO1 in oncogenic and tumor suppressive pathway are well known. Hence, in depth knowledge about FOXO1 associated proteins would shed the light on the molecular mechanisms chiefly involved in their heterogeneity. Discovering activators or stimulators of FOXO1 could be a novel strategy for the treatment and prevention of OSCC and other related cancers.

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Disclosure of conflict of interest

None.

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