

## Editorial

## FOXO3a: A Potential Target in Prostate Cancer

Sanjeev Shukla\*

Department of Urology, Case Western Reserve University,  
USA**\*Corresponding author:** Sanjeev Shukla, Department  
of Urology, Case Western Reserve University, Cleveland,  
Ohio-44106, USA**Received:** May 12, 2014; **Accepted:** May 14, 2014;**Published:** May 16, 2014

## Editorial

Fork head box O transcription factors (FOXO) regulates multiple cellular process, including cell cycle arrest, cell death, DNA damage repair, stress resistance and metabolism [1]. Moreover, inactivation or alteration of FOXO protein is linked to tumorigenesis in the form of breast, prostate, glioblastoma, rhabdomyosarcoma, and leukemic cancers [2]. FOXO family proteins contain four members, FOXO1, FOXO3a, FOXO4, and FOXO6. FOXO1, 3, and 4 are generally expressed ubiquitously, although FOXO1 exhibits greater expression in adipose tissue. Liver expresses the greatest amounts of FOXO3a expression, FOXO4 can be found in skeletal muscle while FOXO6 expression is localized predominantly in the brain [3]. AKT, the PI3K cascade effect or protein, negatively regulates these four members [4]. Evidence suggests oncogenic path way activation involves phosphoinositide-3-kinase/AKT/IKK or RAS/mitogen-activated protein kinase subsequently suppresses FOXO transcriptional activity [2]. Phosphorylation of FOXO proteins at different residues by these pathways ultimately results in inactivation, degradation, and/or nuclear exclusion. Additionally, acetylation, methylation, and ubiquitination may also modulate FOXO3a function. Several anti-cancer drugs, imatinib, paclitaxel, and doxorubicin increase FOXO3a by preventing oncogenic suppression of FOXO protein function [5]. The aim of this review is to highlight the involvement of FOXO3 a in prostate cancer and explore available therapeutic targets modulating FOXO3a signaling.

Various molecular targets regulate FOXO3a and alter effect or down-stream signaling molecules. A crucial event in the FOXO3a cascade is the activation of phosphoinositide 3-kinase (PI3K). Activation of PI3K is a hallmark of many human tumors and promotes cell proliferation and survival by phosphorylation of the serine and threonine kinase Akt on the cytosolic side of the plasma membrane by phosphoinositide-dependent protein kinase 1 (PDK1). Nuclear PDK1 promotes cell proliferation by suppressing FOXO3A-dependent transcription of p27(Kip1) [6]. Reports suggest growth factor-dependent phosphorylation of threonine 308 (Akt-308) by phosphatidylinositol 3-kinase-dependent PDK1 leads to activation of mammalian target of rapamycin (mTOR) complex 1 (TORC1) and stimulation of protein synthesis. Phosphorylation of serine 473 (Akt-473) is catalyzed by mTOR in a second complex (TORC2), and Akt-473 phosphorylates FOXO3a leading to apoptotic inhibition [7]. Both phosphorylation forms of Akt frequently occur in cancer. Further, TORC2 activity is required for progression to prostate cancer in

the context of Pten mutations [7]. FOXO3a also has an antioxidant properties, it prevents damage from reactive oxygen species (ROS) by inducing expression of pro survival (e.g., MnSOD) as well as proapoptotic (e.g., Bim) molecules [8]. Conflicting with the loss of FOXO expression early in carcinogenesis, development of oxidative resistance is associated with increased expression of FOXO3a, NAMPT, and SIR1 proteins [9]. This suggests, in addition to initial suppression of the proposed anti-tumorigenic properties of the FOXOs, survival of established cancer cells after chemotherapeutic exposure requires FOXO mediated ROS responses.

We reported altered expression and modifications of FOXO transcription factor activity plays a role in prostate cancer progression. During prostate cancer progression, increases in Akt activation lead to increased FOXO3a phosphorylation and binding with 14-3-3 (a chaperone Protein), which potentially affect transcriptional activity in an age dependent manner. In particular, a mouse model of prostate cancer, transgenic adenocarcinoma of the mouse prostate (TRAMP) mice mimics the progressive form of human prostate progression. Furthermore, suppression of FOXO3a activity results in accelerated prostate cancer progression in TRAMP mice [10]. Additionally, we have demonstrated FOXO3A activity is negatively regulated by Akt/PKB through post translational modifications. In prostate cancer cells, Akt activation induced increased cytosolic accumulation of FOXO3A and binding to 14-3-3. Accumulated cytosolic FOXO3A correlates to Ser253 phosphorylation and accounted for FOXO3a nuclear exclusion. Expression of a dominant negative Akt in PC-3 cells induced FOXO3A nuclear accumulation and up regulation of MnSOD, a downstream target of FOXO3a. Conversely, expression of stable DU145-Akt exhibit decreased nuclear FOXO3A. Similar findings are found in primary prostate tumor samples, in which marked cytoplasmic accumulation of FOXO3A and 14-3-3 increased with Gleason grade, in contrast to exclusive nuclear accumulation normally seen in benign prostate cells [11]. Similarly, FOXO3a was also found to be regulated by another kinase, the serum/glucocorticoid regulated kinase 1 (SGK1). Enhanced survival, invasiveness, motility, epithelial to mesenchymal transition, and tumor adhesiveness correlated with increased expression of SGK1 [12]. Pharmacological studies of SGK1 inhibition are ongoing however suggests SGK1 inhibits prostate cancer cell growth.

Androgen receptor (AR) is known to promote proliferation of prostate cancer cells. Modulation of FOXO3a expression and phosphorylation effects the expression of AR. Reports suggest FOXO3a induces 5' AR promoter activity by binding to the DNA-binding consensus sequence of AR at the -1290 to -1297 (5'-TTGTTTCA-3') residues upstream of the 5' AR promoter [13]. FOXO function is compromised in androgen-independent prostate cancer cell line LNAI while in the androgen-dependent LNCaP cells FOXO function is intact. Moreover, the FOXO-responsive promoter, 3X-IRS, is also reduced in LNAI cells. Reduced FOXO3a expression coupled to increased FOXO3a phosphorylation coincide with reduced FOXO-responsive promoter activity in the androgen-

independent LNAI cells [14]. Additional studies suggest isoflavone inhibits FOXO3a promoter binding to the AR promoter and increases FOXO3a binding to the p27 (KIP1) promoter resulting in lower AR and p27 (KIP1) expression, inhibition of proliferation, and apoptosis in both androgen-sensitive and -insensitive prostate cancer cells. The anti apoptotic factor, FADD-like interleukin-1beta-converting enzyme (FLICE)-like inhibitory protein (FLIP), is also associated with FOXO3a. Androgen induces expression of FLIP while reduced FLIP expression occurs upon androgen withdrawal preceding apoptosis. A FOXO3a binding site was identified in the FLIP promoter and thus both androgens and FOXO3a effect FLIP transcription. Additionally, FOXO3a binds the AR, suggesting FLIP transcription is in part dependent on an interaction between FOXO and the AR proteins [15].

Apoptosis is one mechanism thought to suppress the development of prostate cancer. Reports suggest astrocyte-elevated gene-1 (AEG-1) is up regulated in several malignancies including clinical prostate cancer tissues and prostate cancer cells. Silencing AEG-1 up regulates FOXO3a activity and induces apoptosis [16]. Promyelocytic leukemia protein (Pml) deficiency causes prostate tumorigenesis. Progressive reduced Pml levels prevents FOXO3a mediated transcription of the proapoptotic factor Bim and the cell cycle molecule p27 (kip1) [17]. Proximal to the Fork head binding element of the p27 (kip1) promoter region, there is a functional association between FOXO3a and c-Myc. This interaction might be one reason for the observed inhibition of FOXO3a-mediated activation of the p27 gene and also by the high aberrant expression of c-Myc in many tumor cells likely contributing to uncontrolled proliferation and invasion tumor phenotypes [18].

Estrogen receptor beta (ER $\beta$ ) is a proapoptotic factor and induces apoptosis early in prostate cancer progression upon binding estrogen. ER $\beta$  activity increases expression of the pro-apoptotic factor p53-upregulated modulator of apoptosis (PUMA), independent of p53 but dependent on FOXO3a [19]. Prostate cancer cells demonstrate FOXO3a directly binds to the promoter of promyelocytic leukaemia zinc finger (PLZF) gene. PLZF protein belongs to the family of Krüppel-like zinc finger proteins. This protein is a transcriptional repressor of cell cycle control, spermatogenesis, and prostate cancer. PTEN the known major player in various cancers regulates PLZF expression through AKT/FOXO3a signaling pathway [20]. TNF- $\alpha$ , is also a potential therapeutic target. In prostate cancer TNF- $\alpha$  induces cell survival and resistance to therapy. Rapamycin of prostate cancer cells stimulates TNF-alpha-dependent apoptosis and illustrates the association between c-Flip promoter activity and FOXO3a activation, which might drive apoptotic induction [21].

Cancer cells in general experience more oxidative stress than normal cells. Introduction of additional ROS mediated damage to cancer cells or suppression of antioxidant capacity may induce cancer cell death. However, this may not be lethal to normal cells capable of maintaining redox homeostasis under exogenous ROS through further adaptive responses. Parthenolide, an agent known to activates NADPH oxidase in PC3 but not PrEC cells and leads to a decrease in reduced thioredoxin, activation of phosphoinositide 3-kinase/Akt, and subsequent FOXO3a phosphorylation, ultimately resulting in the down regulation of the FOXO3a targets, antioxidant enzyme manganese superoxide dismutase and catalase [22].

Apoptotic induction in cancer cells suppresses prostate cancer progression. Resveratrol; a naturally occurring phytopolyphenolic compound, induces apoptosis. Inhibition of FOXO phosphorylation is thought to be the mechanism behind the action of resveratrol and results in nuclear translocation, DNA binding, and transcriptional activity. Prostate cancer cells treated with resveratrol show less PI3K/AKT pathway activity and increased FOXO transcriptional activity. Additionally, resveratrol induced Bim, TRAIL, p27/KIP1, DR4, DR5, and inhibition of cyclin D1 [23]. Asian diets containing soy isoflavones have also been correlated to lower incidence of prostate cancer. The isoflavone concentrate (ISF) significantly increases cyclin-dependent kinase inhibitor p27 (KIP1) and FOXO3A/FKHRL1, in LNCaP cells. ISF down regulates androgen-regulated genes in prostate cancer progression, while at the same time up regulating metabolic genes [24]. Apoptosis induced by methyl-2-cyano-3, 12-dioxooleana-1, 9(11)-dien-28-oate (CDDO-Me), an oleanane synthetic triterpenoid is through inhibition of the Akt/NF- $\kappa$ B/mTOR signaling cascade. CDDO-Me directly inhibit Akt kinase, phosphorylation/inactivation of proapoptotic procaspase-9, Bad, and FOXO3a activities [25]. Additionally, CDDO-Me modulates downstream targets p-Bad and p-FOXO3a, p-S6K1, p-eIF-4E, p-4E-BP1, COX-2, VEGF, and cyclin D1 [26]. Similarly, another compound, 3, 3'-diindolylmethane (DIM) treatment of prostate cancer cells targeted FOXO3a, glycogen synthase kinase-3beta (GSK-3beta), and regulated beta-catenin, inhibited cell proliferation and induced apoptotic cell death [27]. Nutritional grade B-DIM (absorption-enhanced), inhibited FOXO3a binding to the AR promoter induced FOXO3a binding to the p27 (KIP1) promoter. Subsequently, FOXO3a bound to the p27 (KIP1) promoter, resulted in the alteration of AR as well as p27 (KIP1) expression as well as inhibiting cell proliferation and the induction of apoptosis in both androgen-sensitive and -insensitive prostate cancer cells [27]. Moreover, another phytoestrogen; genistein acetylates H3-K9 at the p53 and FOXO3a promoter through the reduction of endogenous SIRT1 activity in prostate cancer cells. However, sulforaphane in combination with TRAIL was more effective in inhibiting markers of angiogenesis and metastasis and activated FOXO3a transcription factor in prostate cancer cells [28]. The synthetic compound, NSC126188 also induces apoptosis of PC-3 cells by interfering with membrane recruitment of Akt, resulting in Akt dephosphorylation and FOXO3a activation, which leads to transcription of RhoB. These results suggest RhoB might be a target gene of FOXO3a and is regulated through Akt signaling [29].

Previously we reported apigenin suppressed prostate tumorigenesis in TRAMP mice through PI3K/Akt/FOXO-signaling pathway. Apigenin-treated TRAMP mice (20 and 50  $\mu$ g/mouse/day, 6 days/week for 20 weeks) exhibited significant decreased tumor volumes of the prostate as well as completely abolish distant organ metastasis. Apigenin-treated mice showed reduced phosphorylation of Akt (Ser473) and FOXO3a (Ser253), which correlate with increased nuclear retention and decreased binding of FOXO3a with 14-3-3. These events lead to reduced proliferation as assessed by Ki-67 and cyclin D1, along with up regulation of FOXO-responsive proteins BIM and p27/Kip1 [30].

Cancer stem cells are thought to have modifications to many of the above mediators of tumorigenesis resulting in reduced response to therapy. Moreover potential involvement of FOXO3a

in different cancer stem cells has been reported; ovarian cancer cells [31], hepatocellular carcinoma cells [32], colon cancer cells [33] and glioblastoma cells [34]. Moreover, prostate cancer tumors with an increased CD133 (+)/CD44 (+) cell subpopulation thought to be prostate cancer progenitor, cells have tumor initiating potential. These progenitors expand under non-adherent, serum-free, sphere-forming conditions and have increased in vitro clonogenic and in vivo tumorigenic potential as well as activated PI3K/AKT signaling. When FOXO3a is knocked down, these prostate tumor cells have increased tumorigenic potential [35]. Therefore, in addition to the above molecular targets further investigations should also focus on targeting the prostate cancer stem cell subpopulation as well.

Based on recent studies and as well as also studies from our group have identified FOXO3a as playing a key role in the development and pathology of prostate cancer. FOXO3a may also be important in the development of prostate cancer progenitor cells. This possibility has driven the focus of research to mechanistically describe how FOXOs and loss of FOXO activity lead to the development of prostate tumors. Recurrence and metastasis are the major process involved in prostate cancer. These processes are supported by a microenvironment enriched with inflammatory mediators and oxidative stress. FOXO3a negatively regulates oxidative stress but at the same time decrease in FOXO3a lead to a microenvironment favoring the proliferation and survival of prostate cancer cells. Effective strategies are necessary to prevent the loss of FOXO3a activity. Therapeutics designed to enhance FOXO3a activity would provide an advantage to prevent prostate cancer progression and to treat prostate cancer.

## Acknowledgment

This work was supported by grants from United States Public Health Services RO3CA166231 to SS.

## References

- Storz P. Forkhead homeobox type O transcription factors in the responses to oxidative stress. *Antioxid Redox Signal*. 2011; 14: 593-605.
- Yang JY, Hung MC. Deciphering the role of forkhead transcription factors in cancer therapy. *Curr Drug Targets*. 2011; 12: 1284-1290.
- Cheng Z, White MF. Targeting Forkhead box O1 from the concept to metabolic diseases: lessons from mouse models. *Antioxid Redox Signal*. 2011; 14: 649-661.
- Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell*. 1999; 96: 857-868.
- Daitoku H, Sakamaki J, Fukamizu A. Regulation of FoxO transcription factors by acetylation and protein-protein interactions. *Biochim Biophys Acta*. 2011; 1813: 1954-1960.
- Kikani CK, Verona EV, Ryu J, Shen Y, Ye Q, Zheng L, et al. Proliferative and antiapoptotic signaling stimulated by nuclear-localized PDK1 results in oncogenesis. *Sci Signal*. 2012; 5: ra80.
- El-Naggar S, Liu Y, Dean DC. Mutation of the Rb1 pathway leads to overexpression of mTOR, constitutive phosphorylation of Akt on serine 473, resistance to anoikis, and a block in c-Raf activation. *Mol Cell Biol*. 2009; 29: 5710-5717.
- Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med*. 2010; 48: 749-762.
- Wang B, Hasan MK, Alvarado E, Yuan H, Wu H, Chen WY. NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response. *Oncogene*. 2011; 30: 907-921.
- Shukla S, Bhaskaran N, MacLennan GT, Gupta S. Deregulation of FoxO3a accelerates prostate cancer progression in TRAMP mice. *Prostate*. 2013; 73: 1507-1517.
- Shukla S, Shukla M, MacLennan GT, Fu P, Gupta S. Deregulation of FOXO3a during prostate cancer progression. *Int J Oncol*. 2009; 34: 1613-1620.
- Lang F, Perrotti N, Stournaras C. Colorectal carcinoma cells--regulation of survival and growth by SGK1. *Int J Biochem Cell Biol*. 2010; 42: 1571-1575.
- Yang L, Xie S, Jamaluddin MS, Altuwajiri S, Ni J, Kim E, et al. Induction of androgen receptor expression by phosphatidylinositol 3-kinase/Akt downstream substrate, FOXO3a, and their roles in apoptosis of LNCaP prostate cancer cells. *J Biol Chem*. 2005; 280: 33558-33565.
- Lynch RL, Konicek BW, McNulty AM, Hanna KR, Lewis JE, Neubauer BL, et al. The progression of LNCaP human prostate cancer cells to androgen independence involves decreased FOXO3a expression and reduced p27KIP1 promoter transactivation. *Mol Cancer Res*. 2005; 3: 163-169.
- Cornforth AN, Davis JS, Khanifar E, Nastiuk KL, Krolewski JJ. FOXO3a mediates the androgen-dependent regulation of FLIP and contributes to TRAIL-induced apoptosis of LNCaP cells. *Oncogene*. 2008; 27: 4422-4433.
- Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, et al. Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. *Oncogene*. 2007; 26: 7647-7655.
- Chatterjee A, Chatterjee U, Ghosh MK. Activation of protein kinase CK2 attenuates FOXO3a functioning in a PML-dependent manner: implications in human prostate cancer. *Cell Death Dis*. 2013; 4: e543.
- Chandramohan V, Mineva ND, Burke B, Jeay S, Wu M, Shen J, et al. c-Myc represses FOXO3a-mediated transcription of the gene encoding the p27(Kip1) cyclin dependent kinase inhibitor. *J Cell Biochem*. 2008; 104: 2091-2106.
- Dey P, Ström A, Gustafsson JA. Estrogen receptor  $\beta$  upregulates FOXO3a and causes induction of apoptosis through PUMA in prostate cancer. *Oncogene*. 2013;.
- Cao J, Zhu S, Zhou W, Li J, Liu C, Xuan H, et al. PLZF mediates the PTEN/AKT/FOXO3a signaling in suppression of prostate tumorigenesis. *PLoS One*. 2013; 8: e77922.
- Cornforth AN, Davis JS, Khanifar E, Nastiuk KL, Krolewski JJ. FOXO3a mediates the androgen-dependent regulation of FLIP and contributes to TRAIL-induced apoptosis of LNCaP cells. *Oncogene*. 2008; 27: 4422-4433.
- Sun Y, St Clair DK, Xu Y, Crooks PA, St Clair WH. A NADPH oxidase-dependent redox signaling pathway mediates the selective radiosensitization effect of parthenolide in prostate cancer cells. *Cancer Res*. 2010; 70: 2880-2890.
- Chen Q, Ganapathy S, Singh KP, Shankar S, Srivastava RK. Resveratrol induces growth arrest and apoptosis through activation of FOXO transcription factors in prostate cancer cells. *PLoS One*. 2010; 5: e15288.
- Rice L, Handayani R, Cui Y, Medrano T, Samedi V, Baker H, et al. Soy isoflavones exert differential effects on androgen responsive genes in LNCaP human prostate cancer cells. *J Nutr*. 2007; 137: 964-972.
- Deeb D, Gao X, Jiang H, Dulchavsky SA, Gautam SC. Oleanane triterpenoid CDDO-Me inhibits growth and induces apoptosis in prostate cancer cells by independently targeting pro-survival Akt and mTOR. *Prostate*. 2009; 69: 851-860.
- Liu Y, Gao X, Deeb D, Gautam SC. Oleanane triterpenoid CDDO-Me inhibits Akt activity without affecting PDK1 kinase or PP2A phosphatase activity in cancer cells. *Biochem Biophys Res Commun*. 2012; 417: 570-575.
- Li Y, Wang Z, Kong D, Murthy S, Dou QP, Sheng S, et al. Regulation of FOXO3a/beta-catenin/GSK-3beta signaling by 3,3'-diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in prostate cancer cells. *J Biol Chem*. 2007; 282: 21542-21550.
- Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, et al. Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells. *Int J Cancer*. 2008; 123: 552-560.

29. Won KJ, Kim BK, Han G, Lee K, Jung YJ, Kim HM, et al. NSC126188 induces apoptosis of prostate cancer PC-3 cells through inhibition of Akt membrane translocation, FoxO3a activation, and RhoB transcription. *Apoptosis*. 2014; 19: 179-190.
30. Shukla S, Bhaskaran N, Babcook MA, Fu P, Maclennan GT, Gupta S. Apigenin inhibits prostate cancer progression in TRAMP mice via targeting PI3K/Akt/FoxO pathway. *Carcinogenesis*. 2014; 35: 452-460.
31. Ning Y, Luo C, Ren K, Quan M, Cao J. FOXO3a-mediated suppression of the self-renewal capacity of sphere-forming cells derived from the ovarian cancer SKOV3 cell line by 7-difluoromethoxyl-5,4'-di-n-octyl genistein. *Mol Med Rep*. 2014; 9: 1982-1988.
32. Xie C, Xie DY, Lin BL, Zhang GL, Wang PP, Peng L, et al. Interferon- $\beta$  gene-modified human bone marrow mesenchymal stem cells attenuate hepatocellular carcinoma through inhibiting AKT/FOXO3a pathway. *Br J Cancer*. 2013; 109: 1198-1205.
33. Ordóñez-Morán P, Irmisch A, Barbáchano A, Chicote I, Tenbaum S, Landolfi S, et al. SPROUTY2 is a  $\beta$ -catenin and FOXO3a target gene indicative of poor prognosis in colon cancer. *Oncogene*. 2014; 33: 1975-1985.
34. Sunayama J, Sato A, Matsuda K, Tachibana K, Watanabe E, Seino S, Suzuki K. FoxO3a functions as a key integrator of cellular signals that control glioblastoma stem-like cell differentiation and tumorigenicity. *Stem Cells*. 2011; 29: 1327-1337.
35. Dubrovskaya A, Kim S, Salamone RJ, Walker JR, Maira SM, García-Echeverría C, et al. The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. *Proc Natl Acad Sci U S A*. 2009; 106: 268-273.