Asian Biomedicine Vol. 7 No. 5 October 2013; 627-634 DOI: 10.5372/1905-7415.0705.221

Original article

The expression and significance of FOXO3a and Jab1 in human ovarian cancer

Mudan Lu, Fei Xu, Yong Wang, Yongxiang Yin, Jingying Xiang, Daozhen Chen
Genetic Laboratory, Wuxi Hospital for Maternal and Child Health Care, Affiliated Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu 214002, China

Background: Ovarian carcinoma represents one of the most insidious and aggressive cancers. Ovarian carcinoma is the most lethal gynecological malignancy. To discover new relevant biomarkers to increase specificity and sensitivity for early diagnosis and prognosis of ovarian cancer is important and urgently needed. FOXO3a possesses a large series function including cellular proliferation, transformation, differentiation, and longevity. Jab1 is also known as subunit 5 (CSN5) of the COP9 signalosome (CSN), a multifunctional protein complex involved in modulating signal transduction, gene transcription, and protein stability.
Objective: To investigate the importance of FOXO3a and Jab1 in ovarian cancer.
Method: Immunohistochemical analysis was performed on formalin-fixed paraffin sections of 46 specimens. Statistical analysis was conducted using the Stat View 5.0 software package.
Result: We found that Foxo3a expression correlates significantly with FIGO disease stage ($p < 0.05$) and lymph node involvement. Jab1 expression correlates significantly with disease stage and lymph node involvement ($p < 0.05$). A multivariate Cox proportional hazard model showed that Foxo3a and Jab1 were the strongest independent predictors of survival ($p < 0.05$), the second predictor being FIGO disease stage and lymph node involvement.
Conclusion: Understanding the precise role of Jab1 and Foxo3a in ovarian cancer progression will not only increase our knowledge of the biology of ovarian cancer but may also enable development of an novel therapeutic strategy via suppression of Jab1.

Keywords: Jab1, FOXO3a, ovarian cancer, prognosis, proliferation

Ovarian carcinoma is one of the most insidious and aggressive cancers and is the most lethal gynecological malignancy [1]. The high mortality rate of women with ovarian cancer is a consequence of the fact that 70%–75% of women with this disease are diagnosed with stage III or IV disease [2]. Thus, discovery of new relevant biomarkers to increase the specificity or sensitivity for early diagnosis and prognosis of ovarian cancer is an important and urgent need. Besides their potential clinical use for diagnosis and prognosis of this disease, study of their pathological function in tumorigenesis or malignancy will not only provide a molecular basis of understanding in fundamental cancer biology, but also may identify potential signaling mediators of the biomarkers in cancer cells for targeting therapy in the future.

Correspondence to: Daozhen Chen and Jingying Xiang, Genetic Laboratory, Wuxi Hospital for Maternal and Child Health Care, Affiliated Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu 214002, China. E-mail: lumudan0527@163.com

The Forkhead family of transcription factors is characterized by the presence of a conserved 100-amino acid DNA binding domain and participates in regulating diverse cellular functions such as apoptosis, differentiation, metabolism, proliferation, and survival [3]. Foxo3a is a substrate of protein kinase Akt, and its transcriptional output is controlled via phosphorylation. In the absence of cellular stimulation and when Akt is inactive, Foxo3a is localized within the nucleus where it performs transcription of target genes. However, upon phosphorylation by Akt at Thr-32, Ser-253, and Ser-315, it binds to 14-3-3 and cannot exert the transcriptional function [4]. Akt phosphorylation also induces nuclear export of FOXO proteins through the nuclear pore complex, which is dependent on 14-3-3 chaperone proteins and the exportin receptor, chromosomal region maintenance protein 1 (CRM1) [5, 6]. Thus, the nuclear, transcription-dependent tumor suppressor function of FOXOs is abolished because of phosphorylation and nuclear exportation.

Jun activation domain-binding protein (Jab1) was initially identified as a coactivator of gene regulatory activator proteins (AP-1) such as c-Jun and JunD [7]. Jab1 is also known as subunit 5 (CSN5) of the COP9 signalosome (CSN), a multifunctional protein complex involved in modulating signal transduction, gene transcription, and protein stability [8, 9]. Recently, several studies have shown that Jab1 is overexpressed in different types of cancer and inversely correlated with $p27^{Kip1}$ expression [10-13]. In addition, overexpression of Jab1 has been associated with poor prognosis [10, 13, 14].

Jab1 plays a significant role in the tumor; however, the expression of Jab1, Foxo3a, and correlation of Jab1 and Foxo3a with ovarian cancer have not been identified. Therefore, in the present study, we examined 46 primary ovarian cancers immunohistochemically and determined the correlation between the levels of these proteins, and various clinical and pathological features including prognosis.

Materials and methods

Patients and tissue samples

Ovarian carcinoma tissues were obtained from 46 patients. All underwent surgical resection without preoperative systemic chemotherapy at the Wuxi Maternal and Child Health Hospital. The main clinical and pathologic variables of the patients are shown in Table 1. The patients' ages ranged from 23 to 71 years (mean = 53.53 ± 22.87). Twenty-one patients were positive for lymph node involvement, 26 were positive for ascites. Histological grades were classified to well-differentiated (grade I; n = 19), moderately-differentiated (grade II; n = 13), and poorly-differentiated tumors (grade III; n = 14). The follow-up time was 5 years for 61 patients ranging from 1 to 60 months. None of the patients received postoperative adjuvant therapy. Tissue samples were immediately processed after surgical removal. For histological examination, all tumorous and surrounding nontumorous tissue portions were fixed in formalin and embedded in paraffin. Informed consent was obtained from all patients.

Immunohistochemistry (IHC)

Tissue sections (4 m) were cut, placed on APES-pretreated slides, deparaffinized, rehydrated through a graded series of alcohol concentrations, and quenched in 3% hydrogen peroxide. Antigen retrieval was performed by microwave heating at high power (750 W) in 10 mM sodium citrate buffer (pH 6.0) for three cycles of 5 min each. After blocking with normal serum for 1 h at room temperature, the sections were incubated overnight at 4 C with anti-human Jab1 mouse monoclonal antibody (diluted 1:100; BD BiosciencesPharMingen, San Diego, CA), anti-Foxo3a rabbit monoclonal antibody (diluted 1:80; Sigma). Negative control slides were also processed in parallel using a nonspecific immunoglobulin IgG (Sigma Chemical Co., St. Louis, MO) at the same concentration as the primary antibody. All slides were processed using a peroxidase–antiperoxidase method (Dako, Hamburg, Germany). Diaminobenzidine was used as the chromogen, and Gill's hematoxylin was used for counterstaining.

All of the immunostained sections were evaluated in a blinded manner without knowledge of the clinical and pathological parameters of the patients. For assessment of Jab1 and Foxo3a, five high-power fields in each specimen were selected randomly, and nuclear staining was examined under high power magnification. More than 500 cells were counted to determine the mean percent, which represented the percentage of immunostained cells relative to the total number of cells. In half of the samples, staining was repeated to avoid possible technical errors, and similar results were obtained from the repeated staining. The evaluation procedures described above were performed by M.D.L. The obtained results were confirmed by other investigator (D.Z.C.) using a multihead microscope, to achieve a consensus.

Statistical analysis

Statistical analysis was performed using the Stat View 5.0 software package. The association between Jab1 and Foxo3a expression and clinicopathological features was analyzed using a χ^2 test. To analyze survival data, Kaplan–Meier curves were constructed, and a log–rank test was performed. Multivariate analysis was performed using a Cox proportional hazards model with $p < 0.05$ considered statistically significant.

Results

The expression of Jab1, Foxo3a, and their correlation with clinicopathologic variables in ovarian cancer

We detected the expression of Jab1 and Foxo3a by IHC in 46 ovarian cancer samples, and the typical case showed that low expression of Jab1 (b) was

correlated with high Foxo3a (d) in the ovarian carcinoma specimen while high expression of Jab1 (a) was correlated with Low Foxo3a (c) in the ovarian carcinoma specimen (**Figure 1**). The results of 46 ovarian carcinomas by IHC are presented in **Figure 1** and summarized in **Table 1**, the mean percent of Foxo3a was 45.26 ± 20.84% and Jab1 was 20.32 ± 12.93%. Based on mean percent, patients were divided into two groups: high Foxo3a expressers (≥45.26%) and low Foxo3a expressers (<45.26%) by Foxo3a (**Table 1**). Foxo3a expression correlates significantly with FIGO disease stage ($p < 0.05$) and lymph node, but there was no relationship between Foxo3a expression and other prognostic factors such as tumor stage, residual disease, and ascites. Furthermore, patients were divided into two groups: high Jab1 expressers (≥20.32**%**) and low Jab1 expressers (<20.32%) by Jab1. Jab1 expression correlates significantly with disease stage and lymph node ($p < 0.05$), and no significant correlation was found between Jab1 expression and other clinicopathologic variables (**Table 1**).

In most specimens, the proportion of Foxo3a-positive tumor cells was greater than the proportion of Jab1-positive tumor cells. An inverse correlation between Foxo3a expression and Jab1 expression was found, with a correlation coefficient of –0.599 ($p > 0.05$; **Figure 2**).

Prognostic significance of Jab1 expression and Foxo3a expression

Concerning survival, only 8 of 27 (29.63% patients in the high Foxo3a-expression group died of disease versus 11 of 19 (57.89%)) in the low Foxo3a-expression group (**Table 2**). Fourteen of 22 (63.64%) patients in the high Jab-expression group died of disease versus 5 of 24 (20.83%) in the low Jab1-expression group (**Table 2**). When all variables were compared separately to survival status, only lymph node involvement ($p < 0.05$), FIGO disease stage ($p < 0.05$), Foxo3a ($p < 0.05$), and Jab1 ($p < 0.05$) significantly influenced survival (Table 3). In univariate analysis, the Kaplan–Meier survival curves did not show any significant relationship between FIGO disease stage ($p = 0.5445$, log–rank test) or other potential prognostic factors ($p > 0.05$, log–rank test) and survival. Conversely, the Kaplan–Meier survival curves of low versus high expressers of Foxo3a (Fig.3A) and Jab1 (**Figure 3B**) showed a highly significant separation. When a multivariate Cox proportional hazard model was constructed (including age, FIGO disease stage, Grade, lymph node metabasis, residual disease, ascites, $p27^{Kip1}$, and HE4 expression). Foxo3a and Jab1 were the strongest independent predictors of survival ($p < 0.05$), the second strongest predictors being FIGO disease stage and lymph node involvement (**Table 3**).

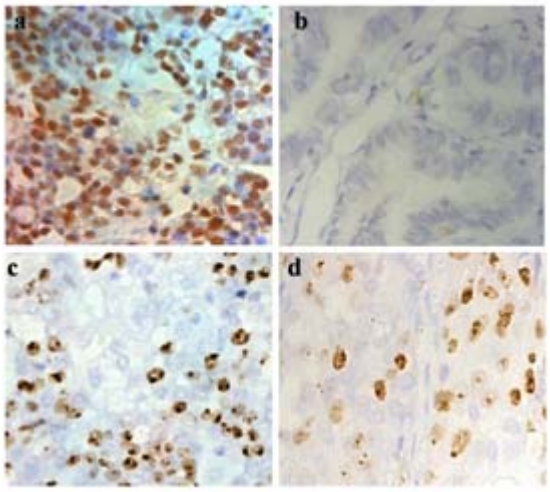

Figure 1. High expression of Jab1 (**a**) is correlated with low expression of FOXO3a (**c**) in the same ovarian cancer specimen while low expression of Jab1 (**b**) is correlated with high expression of FOXO3a (**d**) in the same ovarian cancer specimen. (×40)

Table 1. FOXO3a and JAB1 expression and clinicopathological parameters in 46 ovarian cancer specimens

Variable	Total	FOXO3a			JAB1		
		Low ≤45.26	High >45.26	*p*	Low ≤20.32	High >20.32	*p*
Age(y)							
≤50	18	10	8	0.116	8	10	0.400
>50	28	9	19		16	12	
FIGO disease stage							
I–II	23	5	18	0.007*	19	4	0.000*
III–IV	23	14	9		5	18	
Grade							
G1	19	7	12	0.862	11	8	0.505
G2	13	6	7		5	8	
G3	14	6	8		8	6	
Lymph node							
Negative	25	6	19	0.009*	19	6	0.000*
Positive	21	13	8		5	16	
Residual disease							
≤2 cm	22	11	11	0.251	9	13	0.143
>2 cm	24	8	16		15	9	
Ascites							
Absent	26	9	17	0.293	14	12	0.796
Present	20	10	10		10	10	

GIGO = International Federation of Gynecology and Obstetrics. Statistical analyses were conducted using a Pearson χ^2 test. *p <0.05 was considered significant.

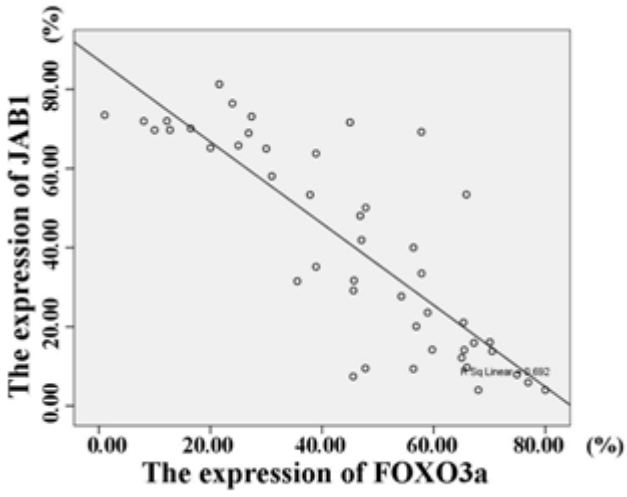

Figure 2. Relationship between Foxo3a and Jab1 expression in ovarian cancer. Scatter plot of Foxo3a and Jab1 with a regression line showing a correlation of the two cell cycle regulators using a Spearman correlation coef cient.

Table 2. Survival status and clinicopathological parameters in 46 ovarian cancer specimens

Variable	Total	Survival status		
		Alive	Dead	*p*
Age(y)				
≤50	18	12	6	0.379
>50	28	15	13	
FIGO disease stage				
I–II	23	18	5	0.007
III–IV	23	9	14	
Grade				
G1	19	14	5	0.219
G2	13	6	7	
G3	14	7	7	
Lymph node				
Negative	25	19	6	0.009*
Positive	21	8	13	
Residual disease				
≤2 cm	22	15	7	0.211
>2 cm	24	12	12	
Ascites				
Absent	26	15	11	0.875
Present	20	12	8	
FOXO3a				
Low expression	19	8	11	0.055
High expression	27	19	8	
JAB1				
Low expression	24	19	5	0.003*
High expression	22	8	14	

Statistical analyses were conducted using a Pearson χ^2 test. *p <0.05 was considered significant.

Discussion

Tumorigenesis, characterized by uncontrolled cell growth and tumor formation, is associated with various alterations in genes or proteins related to regulation of proliferation, cell death, and genomic instability [15]. Thus, identification of genes and their products involved in the molecular events leading to tumorigenesis is critical to developing effective therapeutic strategies. FOXO3a, a member of the FOXO family, has been shown to be a critical tumor suppressor in breast cancer through transcriptional regulation of multiple proteins, including $p21^{Cip1}$, $p27^{Kip1}$, and $cyclinD_1$ [16]. FOXO3a can also physically interact with the p53 protein and activate transcription via p53 sites, suggesting that FOXO3a may cooperate with p53 in suppression of tumorigenesis [17]. Overexpression of FOXO3a can suppress the proliferation and tumorigenesis in athymic mice [18]. In the current study, we found Foxo3a expression correlates significantly with FIGO disease stage ($p < 0.05$) and lymph node involvement, and furthermore Foxo3a was the strongest independent predictor of survival ($p < 0.05$).

FOXO3a concentration is mainly regulated by post-translational mechanisms and one of the key mechanisms is proteolysis by the ubiquitin–proteosome pathway [19]. Akt kinase involved in the translocation of Foxo3a from the nucleus to the cytoplasm through the phosphorylation of specific sites [20]. In addition to the kinase, Jab1 also functions as an adaptor between Foxo3a and CRM1 to induce nuclear export and subsequent degradation. Several lines of evidence have shown that Jab1 is frequently overexpressed in various types of cancers [12]. Here, we report that Jab1 was markedly upregulated in ovarian cancer cells and tissues. Statistical analysis of Jab1 staining revealed that expression of Jab1 was significantly correlated with clinical characteristics of patients, including disease stage and lymph node involvement ($p < 0.05$). These results implicate Jab1 as an oncogenic protein in the development and progression of ovarian cancer.

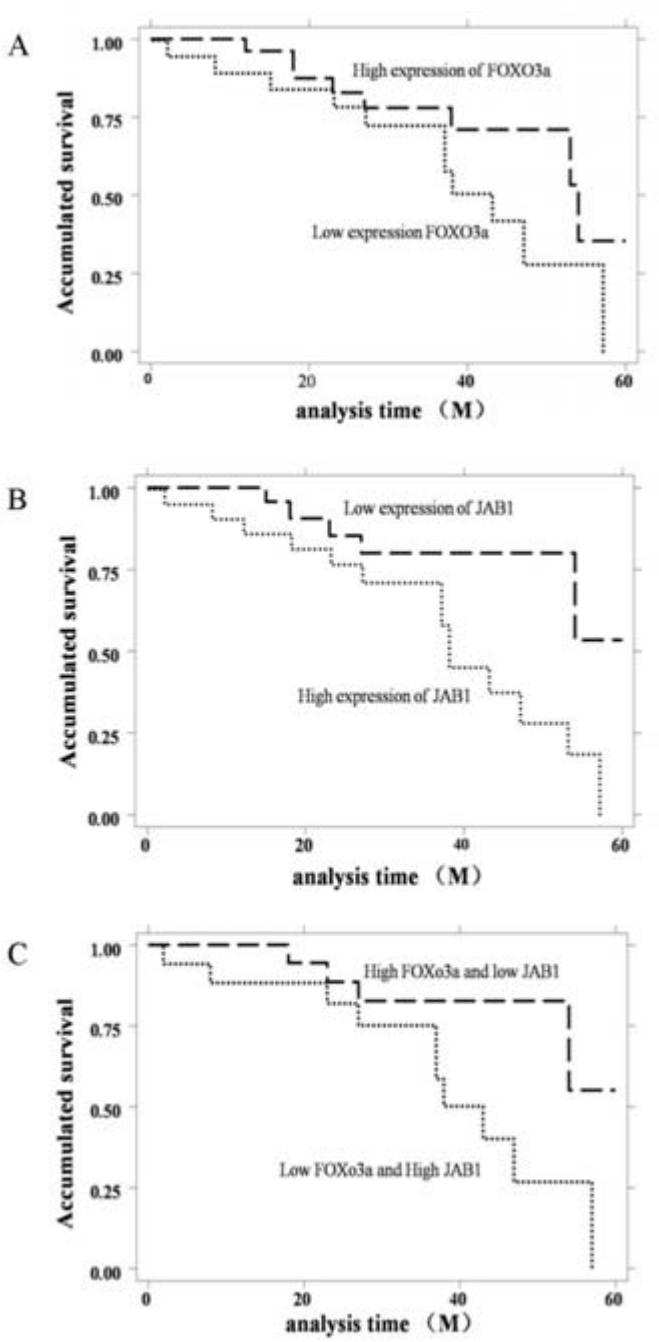

Figure 3. **A)** Kaplan–Meier survival curves for low Foxo3a expression versus high Foxo3a (A) in 46 patients of ovarian cancer showed a highly significant separation ($p < 0.05$, log–rank test). **B)** Kaplan–Meier survival curves for low Jab1 expression versus high Jab1 in 46 patients of ovarian cancer showed a highly significant separation ($p < 0.05$, log–rank test). **C)** Overall survival curves according to Foxo3a/Jab1expression.

Table 3. Contribution of various potential prognostic factors to survival by Cox regression analysis in 46 ovarian cancer specimens

	Hazard ratio	95% Confidence interval	*p*
Age (y)	1.7252	0.6486–4.5883	0.2613
FIGO disease stage	3.1575	1.1315–8.8107	0.0188*
Grade	1.1565	0.6663–2.0072	0.6038
Lymph node	3.8801	1.3677–11.0074	0.0065*
Residual disease	1.7236	0.6721–4.4203	0.2490
Ascites	0.7689	0.3073–1.9241	0.5725
FOXO3a	0.9657	0.9427–0.9893	0.0040*
JAB1	1.0622	1.0156–1.1111	0.0038*

Statistical analyses were conducted using a Pearson χ^2 test. *$p < 0.05$ was considered significant.

In summary, we have shown that Jab1 is evidently overexpressed in ovarian cancers. Moreover, our finding of an inverse correlation between Foxo3a expression and Jab1 expression, with a correlation coefficient of –0.599 illustrates a new mode of action in the molecular mechanism underlying the tumorigenesis of ovarian cancer. Understanding the precise role of Jab1 in ovarian cancer progression will not only increase our knowledge of the biology of ovarian cancer, but may also enable development of a novel therapeutic strategy via suppression of Jab1.

Acknowledgments

This work was supported by a National Natural Scientific Foundation of China Grant (No. 3087299999), Natural Scientific Foundation of Jiangsu Province Grant (No. BK2007023), the Nanjing Medical University Science and Technology Development Fund Accented Term (09NJMUZ48), and the Wuxi Hospital Management Center Medical Technology Development Fund (YGM1023). The authors have no conflict of interest to declare.

References

1. Cannistra SA. Cancer of the ovary. N Engl J Med. 2004; 351:2519-29.
2. Runnebaum IB, Stickeler E. Epidemiological and molecular aspects of ovarian cancer risk. J Cancer Res Clin Oncol. 2001; 127:73-9.
3. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci. 2007; 120:2479-87.
4. 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; 96:857-68.
5. Brunet A, Kanai F, Stehn J, Xu J, Sarbassova D, Frangioni JV, et al. 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. J Cell Biol. 2002; 156:817-28.
6. Van Der Heide LP, Hoekman MF, Smidt MP. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. Biochem J. 2004; 380:297-309.
7. Claret F-X, Hibi M, Dhut S, Toda T, Karin M. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. Nature. 1996; 383:453-7.
8. Chamovitz DA, Segal D. JAB1/CSN5 and the COP9 signalosome. A complex situation. EMBO Rep. 2001; 2:96-101.
9. Schwechheimer C, Deng XW. COP9 signalosome revisited: a novel mediator of protein degradation. Trends Cell Biol. 2001; 11:420-6.
10. Sui L, Dong Y, Ohno M, Watanabe Y, Sugimoto K, Tai Y, et al. Jab1 expression is associated with inverse expression of $p27^{kip1}$ and poor prognosis in epithelial ovarian tumors. Clin Cancer Res. 2001; 7:4130-5.
11. Kouvaraki MA, Rassidakis GZ, Tian L, Kumar R, Kittas C, Claret F-X. Jun activation domain-binding protein 1 expression in breast cancer inversely correlates with the cell cycle inhibitor $p27^{Kip1}$. Cancer Res. 2003; 63:2977-81.
12. Rassidakis GZ, Claret F-X, Lai R, Zhang Q, Sarris AH, McDonnell TJ, et al. Expression of $p27^{Kip1}$ and c-Jun activation binding protein 1 are inversely correlated in systemic anaplastic large cell lymphoma. Clin Cancer Res. 2003; 9:1121-8.
13. Shintani S, Li C, Mihara M, Hino S, Nakashiro K, Hamakawa H. Skp2 and Jab1 expression are associated with inverse expression of $p27^{KIP1}$ and poor prognosis

in oral squamous cell carcinomas. Oncology. 2003; 65: 355-62.
14. Osoegawa A, Yoshino I, Kometani T, Yamaguchi M, Kameyama T, Yohena T, et al. Overexpression of Jun activation domain-binding protein 1 in nonsmall cell lung cancer and its significance in p27 expression and clinical features. Cancer. 2006; 107:154-61.
15. Doerfler W, Hohlweg U, M ller K, Remus R, Heller H, Hertz J. Foreign DNA integration—perturbations of the genome—oncogenesis. Ann N Y Acad Sci. 2001; 945:276-88.
16. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci. 2007; 120:2479-87.
17. Nemoto S, Fergusson MM, Finkel T. Nutrient availability regulates SIRT1 through a Forkhead-dependent pathway. Science. 2004; 306:2105-8.
18. Zou Y, Tsai WB, Cheng CJ, Hsu C, Chung YM, Li PC, et al. Forkhead box transcription factor FOXO3a suppresses estrogen-dependent breast cancer cell proliferation and tumorigenesis. Breast Cancer Res. 2008; 10:R21. doi:10.1186/bcr1872
19. Huang H, Regan KM, Wang F, Wang D, Smith DI, van Deursen JM, et al. Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. Proc Natl Acad Sci USA 2005; 102:1649-54.
20. Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY, et al. Cell. IκB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. 2004; 117: 225-37.