doi:10.4149/neo_2015_112

FBXL10 contributes to the progression of nasopharyngeal carcinoma via involving in PI3K/mTOR pathway

Y. REN[1,‡], L. WU[2,‡], X. LI[1], W. LI[3], Y. YANG[3], M. ZHANG[3,*]

[1]Department of Head and neck surgery, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming 650118, China; [2]Department of Radiology, The Second People's Hospital of Yunnan Province, Kunming 650021, China; [3]Department of Radiation Oncology, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming 650118, China

**Correspondence: zhangming977@126.com*
‡Contributed equally to this work.

Received May 22, 2015 / Accepted July 22, 2015

Our study aimed to investigate whether F-box and leucine-rich repeat protein 10 (FBXL10) may play a pivotal role in nasopharyngeal carcinoma (NPC) development via involving in PI3K/mTOR pathway. We .constructed an FBXL10 expression vector (pcDNA3.1-FBXL10). Then pcDNA3.1-FBXL10 and FBXL10-specific siRNA (siFBXL10) were transfected into human NPC cell line CNE1 and SUNE1 with Lipofectamine ® 2000. Moreover, cells were treated with PI3K inhibitor BEZ235. Besides, MTT assay and flow cytometry were respectively used to explore cell proliferation and apoptosis *in vitro*. Finally, the expression of key proteins involved in PI3K/AKT/mTOR pathway, such as P-AKT, AKT, P-P70, P70, P-Myc and Myc, were determined by western blot. Western blot analysis displayed that FBXL10 was overexpressed and suppressed after transfected by pcDNA3.1-FBXL10 and siFBXL10, respectively. Moreover, cell proliferation in FBXL10 overexpression group gradually increased compared with control group while obviously decreased in siFBXL10 group. Moreover, volume of apoptotic cells significantly increased with knockdown of FBXL10, which was similar with BEZ235 treatment. Besides, knockdown of FBXL10 decreased the expression levels of PI3K/mTOR pathway-related proteins, which was also similar with BEZ235 treatment. Notably, BEZ235 and siFBXL10 treatment induced significant increase of cell apoptosis and decrease of the expression levels of PI3K/mTOR pathway-related proteins than that only treated with siFBXL10. These findings indicate that FBXL10 may play a pivotal role in promoting cell proliferation and inhibiting cell apoptosis in NPC cells via targeting or functioning synergistically with PI3K/mTOR pathway. Knockdown of FBXL10 may be a novel therapeutic strategy for the treatment of NPC.

Key words: nasopharyngeal carcinoma, F-box and leucine-rich repeat protein 10, BEZ235, PI3K/mTOR pathway

Nasopharyngeal carcinoma (NPC) derived from epithelial cells is a high-incidence malignancy, particularly in Southern China [1]. Despite advances in surgical techniques, radiotherapy and chemotherapy, the prognosis of this disease is still poor [2, 3]. The epidemiologic evidence implies that genetic susceptibility, including tumor suppressor genes and proto-oncogenes, has important roles in the stages of NPC carcinogenesis [4]. However, several diagnostic and therapeutic targets have been identified in other cancers and remain not unclear in NPC [5, 6]. Thus, it is urgent to elucidate the molecular mechanism to detect the reliable therapeutic targets of this disease.

The F-box and leucine-rich repeat protein 10 (FBXL10, also known as JHDM1B, KDM2B and Ndy-1) belongs to the JmjC domain-containing histone demethylases and is considered as a tumor accelerator [7]. Penzo *et al.* demonstrated that FBXL10 expression might regulate cancer cell growth in a p53- dependent manner [8]. Tzatsos *et al.* confirmed that FBXL10 was markedly overexpressed in human pancreatic ductal adenocarcinoma (PDAC) and drive the pathogenesis of an aggressive subset of PDAC [9]. Kottakis *et al.* also verified that FBXL10 could function as a master regulator of Polycomb complexes and control cell self-renewal in breast cancer [10]. These findings suggest that FBXL10 contributes to the development of tumor, but regrettably, the roles of FBXL10 in NPC progression have not been fully discussed. Recently, the phosphatidylinositide 3-kinase/mammalian target of rapamycin (PI3K/mTOR) pathways have been shown

to play pivotal roles in tumor growth, counteract apoptosis, and enhance cell migration, thereby are considered as a key potential drug target for novel anticancer therapeutics [11-13]. Huang *et al.* demonstrated that the blockage of mTORC1 could enhance cell apoptosis in NPC and imply mTORC1 signal pathway as a potential target for NPC therapy [14]. Moreover, the dual PI3K/mTOR inhibitors, such as BEZ235 and PF-04691502, have been shown to induce apoptosis and inhibit cell growth in NPC, thus are evaluated as therapeutic drugs in preclinical models of NPC [15, 16]. Thus, PI3K/mTOR pathway may be a key mechanism involved in the progression of NPC. Considering the key roles of FBXL10 in tumor development, it can therefore be hypothesized that FBXL10 may play an important role in NPC progression via targeting PI3K/mTOR pathway.

In the current study, we constructed an FBXL10 expression vector (pcDNA3.1-FBXL10) and utilized FBXL10-specific siRNA to respectively promote and suppress the FBXL10 expression in human NPC cell line CNE1 and SUNE1. Moreover, cells were treated with BEZ235 to evaluate whether FBXL10 can target PI3K/AKT/mTOR pathway, which is previously shown to be a potent and highly selective reversible PI3K pathway inhibitor with antiproliferative and apoptotic activity in cancer [17]. Besides, the roles of FBXL10 in cell proliferation, apoptosis and activation of PI3K/AKT/mTOR pathway were respectively investigated. The objective of our study was to explore whether FBXL10 can play a role in NPC development, as well as whether the role is played via involving in PI3K/mTOR pathway. Our study will provide a new insight into the molecular mechanism of NPC progression and may help to develop novel targets for the treatment of this disease.

Materials and methods

Cell lines and cell culture. Human NPC cell line CNE1 and SUNE1 were purchased from the Cancer Research Institute of Sun Yatsen University (Guangzhou, China) and reagents were purchased from Sigma (St Louis, MO, USA). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (RPMI-1640, Sigma-Alorich Co, St Louis, MO, USA) containing 10% heat-inactivated fetal bovine serum (FBS, Welgene Ltd.), penicillin (50 U/mL), and streptomycin (50 μg/mL), and then incubated in humidified incubator with 5% CO_2 at 37°C.

Vector construction and siRNA transfection. An FBXL10 expression vector (pcDNA3.1-FBXL10) was constructed by sub-cloning the full-length wild-type FBXL10 coding sequence into pcDNA3.1(+), and confirmed by sequencing. The pcDNA3.1-FBXL10 was transfected into cells while the empty construct pcDNA3.1 was transfected as a control. All the cell transfections were conducted using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's protocol. Stable FBXL10 transfectants were generated under G418 (Gibco, Paisley, UK) selection. To further explore the function of FBXL10 in CNE1 and SUNE1s, we used siRNA to silence its expression. The target sequence for FBXL10-specific siRNA (siFBXL10) was AAGUAAGUGAGACUGGAUCUCCACC, and control siRNA (no silencing) were synthesized by GeneP-harma Co (Shanghai, China). Then FBXL10-specific siRNA was transfected into cells. The effectiveness of siRNA silencing was assayed by Western blot analysis.

Cell proliferation assay. The cell proliferative capacity was determined using a 3-(4,5-dimethylthiazol-2-yl)-2 5-diphenyl-2Htetrazolium bromide (MTT) colorimetric assay. Detailed speaking, CNE1 and SUNE1cells were respectively seeded on 96-well plates. Then MTT solution (Sigma, USA) was added to each well at 0 h, 24 h, 48 h, 72 h and 96 h after transfection. Each experiment was performed three times. After incubation for 1-4 h at 37°C, the cell proliferation/viability was calculated based on the absorbance at 570 nm which was measured by an enzyme-labeled instrument (Bio-Rad, USA).

Apoptosis analysis by flow cytometry. Cell apoptosis was detected by annexin V-Cy5 and PI staining (BioVision, K103-25) using FACS analysis. The cells were seeded culture dish and continued to incubate for 24 h. Part of cells in siFBXL10 group and control group were then pre-treated with PI3K inhibitor BEZ235 (1 μM) for 16 h while the other part of the cell was control. Cells in siFBXL10 group and control group were firstly washed with ice-cold phosphate-buffered saline (PBS) and resuspended in annexin V-binding buffer (10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1 mM MgC_{12}, 1.8 mM $CaCl_2$, pH 7.4). Afterwards, annexin V-Cy5 (1:1,000) and 1 mg/ml propidium iodide (PI) solution was added to each. After incubation at room temperature (RT) for 5 min, the cells were determined at 488nm by a FACS Calibur flow cytometer (BD, USA) and analyzed with CELLQuest 3.0 software (BD, USA). In the scatter plot of double variable flow cytometry, the percentage of total apoptotic events (annexin V-Cy5 positive/PI negative) was defined as the sum of early apoptotic cells while the percentage (annexin V-Cy5 positive/PI positive) stands for late apoptotic cells.

Preparation of cell lysates and western blot analysis. The cells were washed with ice-cold PBS and lysed in Mammalian Protein Extraction Reagent (Sigma, C2978). Protein concentrations were examined by Bradford (Bio-Rad, Madrid, Spain). Protein samples with the same concentration per lane were separated on a 10-12 % sodium dodecyl sulfate (SDS)-polyacrylamide gel. Then bands were blotted onto polyvinylidene difluoride membranes. After blocked in PBST (0.1 % triton in PBS), the membrane was probed with primary antibodies overnight at 4°C. The membranes were then incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotech). The immunoreactive protein bands were developed by enhanced chemiluminescence and analyzed by a densitometer. The following primary antibodies were used: P-AKT (S473), AKT, P-P70/S6K (T389), P70/S6K antibodies were obtained from Cell Signaling Technology (Beverly, MA). P-Myc and Myc antibodies were purchased from Roche Molecular Biochemicals. GAPDH antibody and horseradish peroxidase-conjugated second anti-

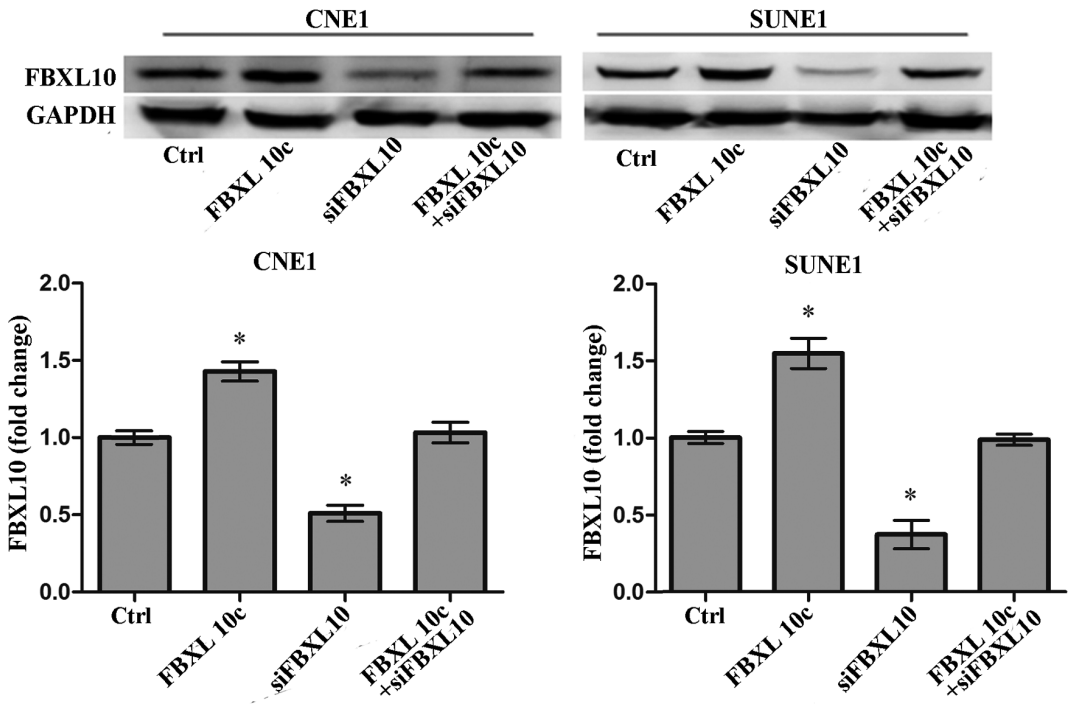

Figure 1. Analysis of the expression level of FBXL10 in human nasopharyngeal carcinoma cell line CNE1 and SUNE1 by western blot. Error bars indicate means ± SD and * indicates significant difference compared with control group (P < 0.05).

body were purchased from Santa Cruz Co (Delaware Avenue, CA, USA). The relative expression level of these proteins was normalized based on the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). To further quantify the results, blots were scanned and analyzed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Statistics analysis. All experiments in our study were repeated three times and measurement data from multiple experiments were presented as the mean ± SD. Statistical analyses were performed using SPSS 17.0 statistical software. T-test (for two groups) or post-hoc Tukey test in one-way ANOVA (for more than three groups) was used to test the comparison between groups. A P-value of < 0.05 was considered to indicate a statistically significant result.

Results

Analysis of the expression level of FBXL10. As shown in Figure 1, the expression level of FBXL10 was determined by western blot. The results showed that FBXL10 in CNE1 and SUNE1 cells was overexpressed and suppressed in FBXL10 overexpression group and siFBXL10 group, respectively. Moreover, there were significant increased and decreased compared with control group (P < 0.05). However, after transfected with both pcDNA3.1-FBXL10 and siFBXL10, the expression level of FBXL10 was no significant difference compared with control (P > 0.05).

Analysis of proliferation rate. The proliferation abilities of CNE1 and SUNE1 cells in an experimental period of 48 h after transfection were measured by MTT assay (Figure 2). The results showed that the cell proliferation/viability of CNE1 cells in siFBXL10 group markedly decreased with the increase of treated time compared with blank group (P < 0.05). Meanwhile, the cell proliferation/viability of CNE1 cells in FBXL10 overexpression group gradually increased with the increase of treated time compared with blank group although the differences did not reach significance (P > 0.05). Similarly, the cell proliferation/viability of SUNE1 cells in siFBXL10 group obvisouly decreased with the increase of treated time compared with blank group while increased in FBXL10 overexpression group (P < 0.05).

Detection of cell apoptosis. The results of FACS analysis displayed that the percentage of apoptotic cells. As shown in Figure 3A, compared with cells in control group, the percentage of apoptotic CNE1 cells significantly increased after knockdown of FBXL10 by siRNA, treated by BEZ235, or both. Moreover, the percentage of apoptotic CNE1 cells significantly increased after treated with BEZ235 + siFBXL10 than only treated with siFBXL10 (P < 0.05). Besides, similar results were obtained in SUNE1 cells (Figure 3B).

Role of FBXL10 in PI3K/AKT/mTOR pathway. In order to further verify the relationship of FBXL10 with PI3K/mTOR pathway, the expression levels of key molecules involved in PI3K/AKT/mTOR pathway were determined by western

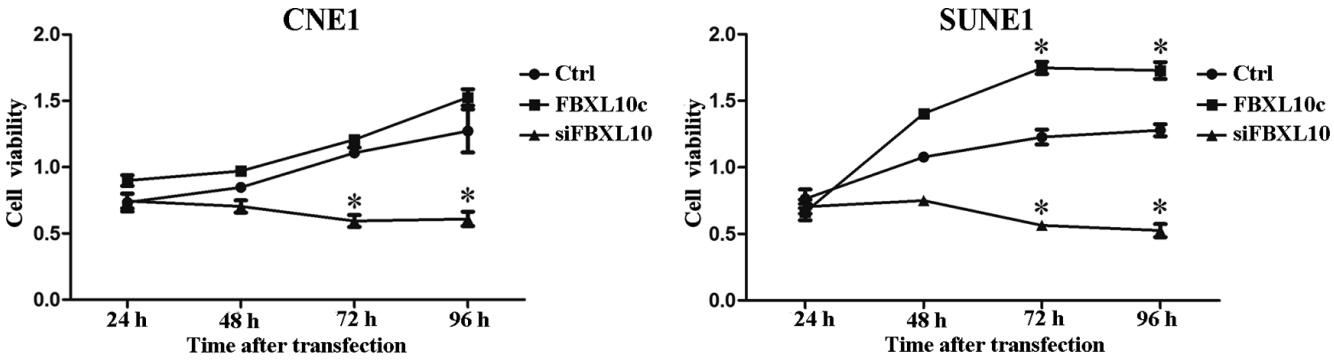

Figure 2 The proliferation/viability of human nasopharyngeal carcinoma cell line CNE1 and SUNE1 in different groups at different times after transfection.

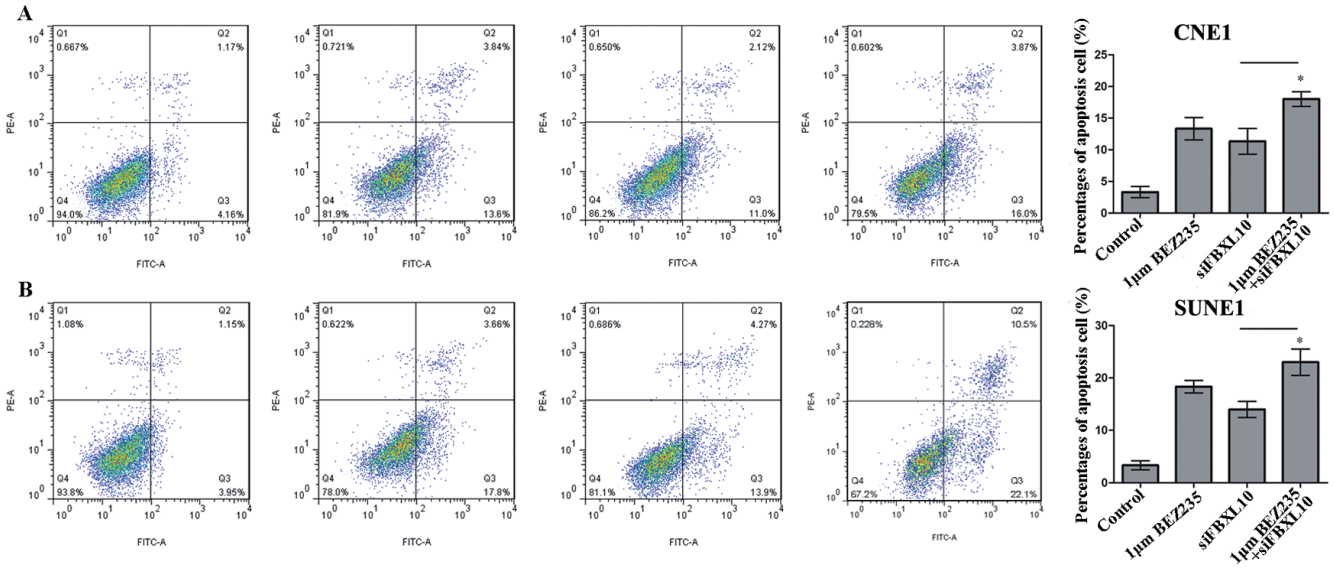

Figure 3. Flow cytometric analysis of cell apoptosis in human nasopharyngeal carcinoma cell line CNE1 (A) and SUNE1 (B) after treated by BEZ235, siFBXL10 and both. Error bars indicate means ± SD and * indicates significant difference compared with control group ($P < 0.05$).

blot (Figure 4). The result showed that the expression levels of P-AKT, P-P70 and P-Myc in BEZ235 group decreased compared that in control group, and the expression levels of P-AKT, AKT, P-P70, P70, P-Myc and Myc in siFBXL10 group decreased. Meanwhile, the expression levels of all these proteins in BEZ235 + siFBXL10 group decreased than that in siFBXL10 group.

Discussion

PI3K/mTOR pathway is shown to be strongly involved in NPC development, and FBXL10 is identified as a key molecule in tumor development. However, the relationships of FBXL10 with PI3K/mTOR and NPC development remain unclear. In the present study, FBXL10 overexpression promoted cell proliferation both in human NPC cell line CNE1 and SUNE1 while knockdown of FBXL10 inhibited. Moreover, the percentage of apoptotic cells significantly increased with knockdown of FBXL10 by siRNA, which was similar with the results after treated by BEZ235. Besides, the expression levels of key proteins involved in PI3K/mTOR pathway decreased with knockdown of FBXL10, which also was similar with the results after treated by BEZ235. Notably, significant increase of cell apoptosis and marked decrease of the expression levels of PI3K/mTOR pathway-related proteins were observed after treated with BEZ235 + siFBXL10 than only treated with siFBXL10. Therefore, the role of FBXL10 and the relationship of FBXL10 with PI3K/mTOR pathway merit further discussion.

MTT assay of cell viability is a good method for assessing the poliferative effects of key factors [18]. In our study, we firstly used this method to evaluate the effects of overexpression and knockdown of FBXL10 on cell proliferation. The results showed that FBXL10 played a positive role in cell

proliferation in NPC cells and were in line with previous findings. For example, He *et al.* verified that FBXL10 was implicated in tumorigenesis and promoted cell proliferation in primary mouse embryonic fibroblasts [19]. Konuma *et al.* demonstrated that force expression of the FBXL10 could maintain self-renewal in hematopoietic stem cells [20]. In addition, FBXL10 is identified as an NF-kB-dependent anti-apoptotic protein, which could regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced apoptosis in various human cancer cells [21]. TRAIL may induce apoptosis in NPC cells via inhibiting the PI3K/Akt signaling pathway [22]. Moreover, Fukuda *et al.* also confirmed that FBXL10 deficiency accelerated neural progenitor cell death in embryonic neural development [23]. In our study, knockdown of FBXL10 contributed to the significantly increased percentage of apoptotic cells, indicating FBXL10 expression could inhibit cell apoptosis in NPC cells. Taken together, we speculate that FBXL10 may contribute to NPC development via promoting cell proliferation and inhibiting cell apoptosis.

In addition, BEZ235 is previously shown to be a potent inhibitor to selectively inhibit PI3K/mTOR pathway with apoptotic activity in NPC [24]. Our results showed that increased apoptotic cells after knockdown of FBXL10 was similar with the results after treated by BEZ235, suggesting that FBXL10 might play a role in NPC development via involving in PI3K/mTOR pathway. In order to further verify the relationship of FBXL10 with PI3K/mTOR pathway, we compared the expression levels of PI3K/mTOR pathway-related proteins after treated with BEZ235, siFBXL10 and both. Similar results were also obtained that knockdown of FBXL10 markedly decreased the expression levels of PI3K/mTOR pathway-related proteins, such as P-AKT, AKT, P-P70, P70, P-Myc and Myc, which was similar with the results after treated by BEZ235. Notably, BEZ235 and siFBXL10 treatment induced significant increase of cell apoptosis and marked decrease of the expression levels of these proteins than that only induced by siFBXL10, implying that FBXL10 may play a key role in NPC development via targeting or functioning synergistically with PI3K/mTOR pathway.

Activation of AKT is believed to protect taxol-induced apoptosis in colon cancer cells via PI3K/AKT pathway [25]. Overexpression of P-AKT is thought to correlate with phosphorylation of epidermal growth factor receptor (EGFR) in NPC [26]. Zhu *et al.* verified that EGFR tyrosine kinase inhibitor AG1478 could inhibit cell proliferation and arrest cell cycle in NPC cells [27]. Moreover, the expression levels of P-P70 and P70 are analyzed to verify the growth-inhibitory activity of NVP-BGT226, a PI3K/mTOR dual inhibitor, against human head and neck cancer cells [28]. Esteva *et al.* demonstrated that the status of P-AKT and P-P70 correlated with trastuzumab response in patients with HER2-positive metastatic breast cancer, suggesting the potential of PI3K pathway activation as targeted therapies [29]. Besides, C-MYC is believed to function as an oncogene to stimulae cell proliferation, elicit apoptosis and change the sensitivity of cancer cells to chemotherapy [30]. Shepherd *et al.* observed that PI3K/mTOR inhibition may cause upregulation of c-MYC [31]. In our study, the expression level of these proteins decreased with knockdown of FBXL10 (Figure 4). It can therefore be speculated that FBXL10 may target or function synergistically with PI3K/mTOR pathway in human NPC cell lines. However, more functional evidences to verify whether FBXL10 contributes to NPC development via targeting or functioning synergistically with PI3K/mTOR pathway are still needed to be provided.

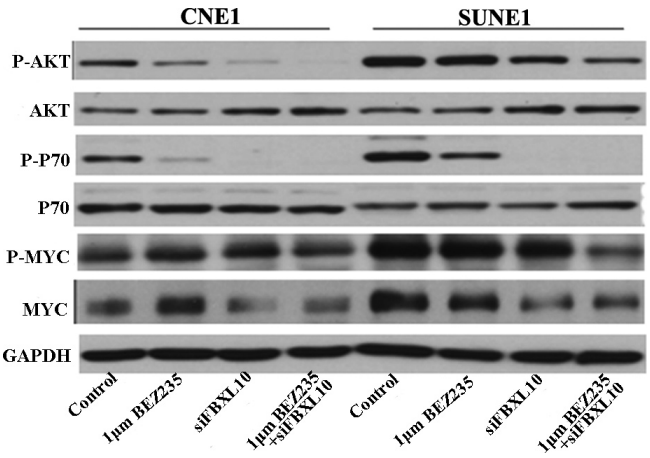

Figure 4. Western blot analysis of the expression level of P-AKT, AKT, P-P70, P70, P-Myc and Myc in human nasopharyngeal carcinoma cell line CNE1 and SUNE1 after treated by BEZ235, siFBXL10 and both. The relative expression level of these proteins was normalized based on the expression level of GAPDH.

In conclusion, FBXL10 expression may play a pivotal role in promoting cell proliferation and inhibiting cell apoptosis in NPC cells via targeting or functioning synergistically with PI3K/mTOR pathway. Knockdown of FBXL10 may be a novel therapeutic strategy for the treatment of NPC.

Acknowledgements: Supported by Yunnan National Natural Science Foundation (No. 2011FB206).

References

[1] LI J, GUO Y, TANG Q, LI S, ZHANG X et al. Prospective study of sensorineural hearing loss following radiotherapy for nasopharyngeal carcinoma. The Journal of Laryngology & Otology 2010; 124: 32–36. http://dx.doi.org/10.1017/S0022215109991435

[2] ALI H, AL-SARRAF M AND ARBOR N. Chemotherapy in advanced nasopharyngeal cancer. Oncology-Huntington 2000; 14: 1223–1231.

[3] RAN Y, WU S and YOU Y. Demethylation of E-cadherin gene in nasopharyngeal carcinoma could serve as a potential therapeutic strategy. Journal of biochemistry 2011; 149: 49–54. http://dx.doi.org/10.1093/jb/mvq128

[4] CHOW S, WANG J, CHUANG S, CHANG Y, CHU W et al. Resveratrol-induced p53-independent apoptosis of human nasopharyngeal carcinoma cells is correlated with the down-regulation of ΔNp63. Cancer gene therapy 2010; 17: 872–882. http://dx.doi.org/10.1038/cgt.2010.44

[5] LOVATO A, ZHAO T, SUN Q and WITCHER M. Poly (ADP-ribose) glycohydrolase (PARG) is a novel therapeutic target in breast cancer. Cancer Research 2013; 73: 4378–4378. http://dx.doi.org/10.1158/1538-7445.AM2013-4378

[6] YOU Y, YANG W, QIN X, WANG F, LI H et al. ECRG4 acts as a tumor suppressor and as a determinant of chemotherapy resistance in human nasopharyngeal carcinoma. Cellular Oncology 2015; 1–10. http://dx.doi.org/10.1007/s13402-015-0223-y

[7] UEDA T, NAGAMACHI A, TAKUBO K, YAMASAKI N, MATSUI H et al. Fbxl10 overexpression in murine hematopoietic stem cells induces leukemia involving metabolic activation and upregulation of Nsg2. Blood 2015; blood-2014–2003-562694.

[8] PENZO M, CASOLI L, POLLUTRI D, SICURO L, CECCARELLI C et al. JHDM1B expression regulates ribosome biogenesis and cancer cell growth in a p53 dependent manner. International Journal of Cancer 2015; 136: E272-E281. http://dx.doi.org/10.1002/ijc.29240

[9] TZATSOS A, PASKALEVA P, FERRARI F, DESHPANDE V, STOYKOVA S et al. KDM2B promotes pancreatic cancer via Polycomb-dependent and-independent transcriptional programs. The Journal of clinical investigation 2013; 123: 727. http://dx.doi.org/10.1172/jci64535

[10] KOTTAKIS F, FOLTOPOULOU P, SANIDAS I, KELLER P, WRONSKI A et al. NDY1/KDM2B functions as a master regulator of Polycomb complexes and controls self-renewal of breast cancer stem cells. Cancer Research 2014; 74: 3935–3946. http://dx.doi.org/10.1158/0008-5472.CAN-13-2733

[11] YAP TA, GARRETT MD, WALTON MI, RAYNAUD F, DE BONO JS et al. Targeting the PI3K–AKT–mTOR pathway: progress, pitfalls, and promises. Current opinion in pharmacology 2008; 8: 393–412. http://dx.doi.org/10.1016/j.coph.2008.08.004

[12] COURTNEY KD, CORCORAN RB and ENGELMAN JA. The PI3K pathway as drug target in human cancer. Journal of Clinical Oncology 2010; 28: 1075–1083. http://dx.doi.org/10.1200/JCO.2009.25.3641

[13] ENGELMAN JA, LUO J and CANTLEY LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nature Reviews Genetics 2006; 7: 606–619. http://dx.doi.org/10.1038/nrg1879

[14] HUANG X-M, DAI C-B, MOU Z-L, WANG L-J, WEN W-P et al. Overproduction of cyclin D1 is dependent on activated mTORC1 signal in nasopharyngeal carcinoma: implication for therapy. Cancer letters 2009; 279: 47–56. http://dx.doi.org/10.1016/j.canlet.2009.01.020

[15] WONG CH, LOONG HH, HUI CW, LAU CP, HUI EP et al. Preclinical evaluation of the PI3K-mTOR dual inhibitor PF-04691502 as a novel therapeutic drug in nasopharyngeal carcinoma. Investigational new drugs 2013; 31: 1399–1408. http://dx.doi.org/10.1007/s10637-013-0007-z

[16] MA BB, LUI VW, HUI CW, LAU CP, WONG CH et al. Preclinical evaluation of the mTOR–PI3K inhibitor BEZ235 in nasopharyngeal cancer models. Cancer letters 2014; 343: 24–32. http://dx.doi.org/10.1016/j.canlet.2013.09.007

[17] BURRIS H, RODON J, SHARMA S, HERBST RS, TABERNERO J et al. First-in-human phase I study of the oral PI3K inhibitor BEZ235 in patients (pts) with advanced solid tumors. ASCO Annual Meeting Proceedings 2010; 28: 3005.

[18] VEGA-AVILA, ELISA PUGSLEY, MICHAEL K. An overview of colorimetric assay methods used to assess survival or proliferation of mammalian cells. Proc West Pharmacol Soc 2011; 54: e4.

[19] HE J, KALLIN EM, TSUKADA Y-I and ZHANG Y. The H3K36 demethylase Jhdm1b/Kdm2b regulates cell proliferation and senescence through p15Ink4b. Nature structural & molecular biology 2008; 15: 1169–1175. http://dx.doi.org/10.1038/nsmb.1499

[20] KONUMA T, NAKAMURA S, MIYAGI S, NEGISHI M, CHIBA T et al. Forced expression of the histone demethylase Fbxl10 maintains self-renewing hematopoietic stem cells. Experimental hematology 2011; 39: 697–709. e695.

[21] GE R, WANG Z, ZENG Q, XU X and OLUMI AF. F-box protein 10, an NF-κB-dependent anti-apoptotic protein, regulates TRAIL-induced apoptosis through modulating c-Fos/c-FLIP pathway. Cell Death & Differentiation 2011; 18: 1184–1195. http://dx.doi.org/10.1038/cdd.2010.185

[22] LI S-S, YANG S, WANG S, YANG X-M, TANG Q-L et al. Latent membrane protein 1 mediates the resistance of nasopharyngeal carcinoma cells to TRAIL-induced apoptosis by activation of the PI3K/Akt signaling pathway. Oncology reports 2011; 26: 1573–1579.

[23] FUKUDA T, TOKUNAGA A, SAKAMOTO R and YOSHIDA N. Fbxl10/Kdm2b deficiency accelerates neural progenitor cell death and leads to exencephaly. Molecular and Cellular Neuroscience 2011; 46: 614–624. http://dx.doi.org/10.1016/j.mcn.2011.01.001

[24] KONSTANTINIDOU G, BEY EA, RABELLINO A, SCHUSTER K, MAIRA MS et al. Dual phosphoinositide 3-kinase/mammalian target of rapamycin blockade is an effective radiosensitizing strategy for the treatment of non–small cell lung cancer harboring K-RAS mutations. Cancer Research 2009; 69: 7644–7652. http://dx.doi.org/10.1158/0008-5472.CAN-09-0823

[25] LIU Y, CHEN L, KO T, FIELDS A and THOMPSON E. Evi1 is a survival factor which conveys resistance to both TGFβ-and taxol-mediated cell death via PI3K/AKT. Oncogene 2006; 25: 3565–3575. http://dx.doi.org/10.1038/sj.onc.1209403

[26] YIP WK, LEONG VCS, ABDULLAH MA, YUSOFF S and SEOW HF. Overexpression of phospho-Akt correlates with phosphorylation of EGF receptor, FKHR and BAD in nasopharyngeal carcinoma. Oncology reports 2008; 19: 319–328. http://dx.doi.org/10.3892/or.19.2.319

[27] ZHU X-F, LIU Z-C, XIE B-F, LI Z-M, FENG G-K et al. EGFR tyrosine kinase inhibitor AG1478 inhibits cell proliferation and arrests cell cycle in nasopharyngeal carcinoma cells. Cancer letters 2001; 169: 27–32. http://dx.doi.org/10.1016/S0304-3835(01)00547-X

[28] CHANG K-Y, TSAI S-Y, WU C-M, YEN C-J, CHUANG B-F et al. Novel phosphoinositide 3-kinase/mTOR dual inhibitor,

NVP-BGT226, displays potent growth-inhibitory activity against human head and neck cancer cells in vitro and in vivo. Clinical Cancer Research 2011; 17: 7116–7126. http://dx.doi.org/10.1158/1078-0432.CCR-11-0796

[29] ESTEVA FJ, GUO H, ZHANG S, SANTA-MARIA C, STONE S et al. PTEN, PIK3CA, p-AKT, and p-p70S6K status: association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. The American journal of pathology 2010; 177: 1647–1656. http://dx.doi.org/10.2353/ajpath.2010.090885

[30] LIAO DJ, THAKUR A, WU J, BILIRAN H and SARKAR FH. Perspectives on c-Myc, Cyclin D1, and their interaction in cancer formation, progression, and response to chemotherapy. Critical Reviews™ in Oncogenesis 2007; 13:

[31] SHEPHERD C, BANERJEE L, CHEUNG CW, MANSOUR MR, JENKINSON S et al. PI3K/mTOR inhibition upregulates NOTCH-MYC signalling leading to an impaired cytotoxic response. Leukemia 2013; 27: 650–660. http://dx.doi.org/10.1038/leu.2012.285