

Original Article

BAF155 inhibits proliferation and migration by up-regulation of p16 and inactivation of PI3K/AKT and Wnt/ β -catenin pathways in PC3 cells

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Abstract: Recent studies have established that BAF155 possess tumor-suppressor activity in human colorectal cancer SNUC2B and human ovarian carcinoma SKOV3 cells. However, the function of BAF155 in human prostate cancer PC3 cells has not been well analyzed. Therefore, the aim of our study was to examine the status of BAF155 in PC3 cells, and explore the mechanism of BAF155 expression loss in PC3. Cell proliferation (MTT) assay, apoptosis assay and migration assay were respectively used to explore the viability, apoptosis and migration of PC3 cells. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed to confirm the mRNA expression level of BAF155. Western blot analysis was performed to determine the protein expression levels of BAF155, p16, and key kinases involved in PI3K/AKT and Wnt/ β -catenin pathways. We identified the BAF155 was completely lost in PC3 cells compared with normal cells. Compared with control, over-expression of exogenous BAF155 could inhibit proliferation and migration while promoting apoptosis of PC3 cells ($P < 0.05$), which could be restored to the normal level by silencing of exogenous BAF155. The further studies on protein expression implied that over-expression of BAF155 markedly up-regulated the expression of p16 while down-regulating the expressions of key kinases involved in PI3K/AKT and Wnt/ β -catenin pathways when compared with that in control ($P < 0.05$). The effects of BAF155 over-expression on proteins could be reversed by silencing of exogenous of BAF155. In conclusion, over-expression of BAF155 could inhibit proliferation and migration while inducing apoptosis of PC3 cells through up-regulating p16 and inactivating PI3K/Akt and Wnt/ β -catenin pathways.

Keywords: BAF155, PC3 cells, p16, PI3K/Akt, Wnt/ β -catenin

Introduction

As one of the most common malignant tumors, prostate cancer (CaP) is reported as the sixth leading cause of cancer-related mortality in men, accounting for 903,500 new diagnoses and 258,400 deaths every year all over the world. Moreover, the case fatality rate of CaP ranks second in all male's cancers, only less than lung cancer. In china, the changes of living habits, diet and age structure make the incidence rate of CaP become increasing year by year.

The yeast switch in mating type/sucrose non fermentation (SWI/SNF) complex with strong conservation in yeast, *Drosophila* and mammals is first discovered in *S. cerevisiae* and

made up of approximately 10-12 components [1]. The complex is an evolutionally conserved and ATP-dependent multiple-subunit chromatin remodeling complex. Previous studies have shown that the SWI/SNF complex plays an important role in various cell biology processes, such as cell cycle, tumor formation and apoptosis [2-4]. The core subunit of SWI/SNF (BRG1 or BRM) has the ATPase activity, which could change the nucleosome structure by the energy of ATP hydrolysis and thus to alter the accessibility of DNA for the transcriptional factors, resulting in regulation of gene expression [5-7]. Some of the SWI/SNF complex subunits, including ARID1A, BRG1/SMARCA4, SNF5/INI1 and PBRM, are tumor suppressor genes. The mechanism of cancer development involving the SWI/SNF complex remains unknown and

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becomes a hot research area. In special, the cancer development induced by SWI/SNF loss may be related to DNA repair, transcription, cell cycle regulation and nucleosome positioning.

BRG1-associated factor (BAF) 155, one member of SWI/SNF complexes, is found in cyclin E complexes and is phosphorylated by cyclin E-associated kinase [8]. The BAF155 and BAF170 proteins are highly homologous and exist either as homodimers (BAF155/155 or BAF170/170) or as heterodimers (BAF155/170) through a leucine zipper motif in the cell [1]. BAF155 contains two highly conserved motifs that are found in chromatin associated proteins. SWIRM (Swi3, Rsc8, and Moria), one domain of BAF155, is believed to adopt an α -helical structure and mediate specific protein-protein interaction [9]. The other motif, termed the SANT (Swi3, Ads2, N-coR, and TFIIB) domain [10], is believed to function as a histone tail binding module [11]. The role of BAF155 in tumorigenesis remains unclear. Since the BAF155 is located on chromosome 3p221.31, which contains some suspected tumor suppressor genes such as FUS1 and SEM3B, the loss of BAF155 might contribute to tumor development [12]. However, several researchers have found increased expression of BAF155 mRNA in prostate cancer, colorectal cancer and cervical intraepithelial neoplasia [13-16].

Recent research showed two carcinoma cell lines lacking BAF155 protein: human colorectal carcinoma SNUC2B cell line and human ovarian carcinoma SKOV3 cell line [17]. Therefore, loss expression of BAF155 might be implicated in tumor development. In the present study, we sought to evaluate the role of BAF155 in human CaP PC3 cells.

Materials and methods

Cell culture

PC3, SNUC2B and Hela cells were cultured in a 5% CO₂ incubator at 37°C with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) except SNUC2B, whose concentration of FBS was 20%. All cell lines were originally obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The mycoplasma contamination of all the cells was tested to be negative before experiments.

Plasmids and small interfering RNA (siRNA) transfection

The over-expression vector of BAF155 (pc-BAF155) was constructed by sub-cloning the full-length coding sequence of wild-type BAF155 into pcDNA3.1 (+), and confirmed by sequencing. The empty pcDNA3.1 was transfected into cells as a control. Specific siRNA against BAF155 (si-BAF155) was synthesized by GenePharma Co., Ltd. (Shanghai, China). Cell transfection was conducted using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Stable transfection was generated under G418 (Gibco, Paisley, UK) selection as described [18].

MTT assay

The cell viability was determined using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay according to standard method described before [19]. Each experiment was performed three times.

Apoptosis assay

Apoptosis analysis was performed to identify and quantify the apoptotic cells by using Annexin V-FITC/PI apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). The cells (100,000 cells/well) were seeded in a 6-well plate. Transfected cells were washed twice with cold PBS and resuspended in binding buffer. The adherent and floating cells were combined and treated according to the manufacturer's instruction, followed by measurement with flow cytometer (Beckman Coulter, Miami, FL, USA) to differentiate apoptotic cells (Annexin-V positive and PI-negative) from necrotic cells (Annexin-V and PI-positive).

Migration assay

Cell migration was determined by using a modified two-chamber migration assay with a pore size of 8 μ m. For migration assay, cells suspended in 200 μ l of serum-free medium were seeded on the upper compartment of 24-well Transwell culture chamber, and 600 μ l of complete medium was added to the lower compartment. After incubation for 12 h at 37°C, cells were fixed with methanol. Non-traversed cells were carefully removed from the upper surface of the filter with a cotton swab. Traversed cells

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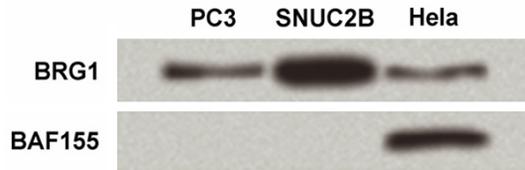


Figure 1. The expression of BAF155 is lost in PC3 cells. HeLa cells and SNUC2B cells were positive control group and negative control group, respectively. The expression of BAF155 was determined by Western blot analysis. BAF, BRG1-associated factor.

on the lower side of the filter were stained with crystal violet and counted.

qRT-PCR

Total RNA was isolated from transfected cells by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and DNaseI (Promega, Madison, WI, USA). Reverse transcription was performed by using the Multiscribe RT kit (Applied Biosystems, Foster City, CA, USA) and random hexamers or oligo (dT). The reverse transcription conditions were 10 min at 25°C, 30 min at 48°C, and a final step of 5 min at 95°C. The sequences of the primers were as follows, BAF155 forward primer: 5'-TCC AAT CTG TGC TGC CCA TC-3', reverse primer: 5'-GAT GCT TCT AGG GGT GAG CC-3'. GAPDH forward primer: 5'-GCA CCG TCA AGG CTG AGA AC-3', reverse primer: 5'-TGG TGA AGA CGC CAG TGG A-3'.

Western blot analysis

The protein used for Western blotting was extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The Western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. Primary antibodies against BAF155 (ab126180), BRG1 (ab110641), p16 antibody (ab51243), Wnt3a (ab28472), Wnt5a (ab72583), β -catenin (ab16051) phosphatidylinositol-3-kinase (PI3K, ab189403), phosphorylated PI3K (p-PI3K, ab182651) (all from Abcam, Cambridge, UK), AKT (9272), phosphorylated AKT (p-AKT, 4060) (both from Cell Signaling Technology, Beverly, MA, USA) and GAPDH (G9545, Sigma, St Louis, MO, USA) were incubated with the polyvinylidene difluoride (PVDF) membrane at 4°C overnight, followed by wash and incubation with

secondary antibodies marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the PVDF membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μ l Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented as the mean \pm SD. Statistical analyses were performed using Graphpad statistical software. The *P*-values were calculated using a one-way analysis of variance (ANOVA). A *P*-value of < 0.05 was considered to indicate a statistically significant result.

Results

The expression of BAF155 is lost in PC3 cells

As shown in **Figure 1**, the expression of BAF155 was lost in PC3 and SNUC2B cells. The expression of BRG1 in SNUC2B cells was almost triple of that in HeLa cells, however, there was nearly no difference between PC3 and HeLa cells.

The expression level of BAF155 after transfection

As shown in **Figure 2**, the mRNA and protein expression level of BAF155 were both significantly increased in cells transfected with pc-BAF155 compared with that of control group ($P < 0.05$). The increase of BAF155 was reversed by additional transfection of si-BAF155, resulting in marked decrease of BAF155 expression compared with cells transfected with pc-BAF155 alone ($P < 0.01$).

BAF155 inhibits the proliferation of PC3 cells

As shown in **Figure 3**, the cell viability of BAF155 over-expressed cells was inhibited compared with that in control group. However, PC3 cell proliferation was restored to the normal level by knockdown of BAF155.

BAF155 promotes the apoptosis of PC3 cells

As shown in **Figure 4**, the cell apoptosis of BAF155 over-expressed cells was significantly increased compared with that in control group ($P < 0.01$). However, the apoptosis of cells co-

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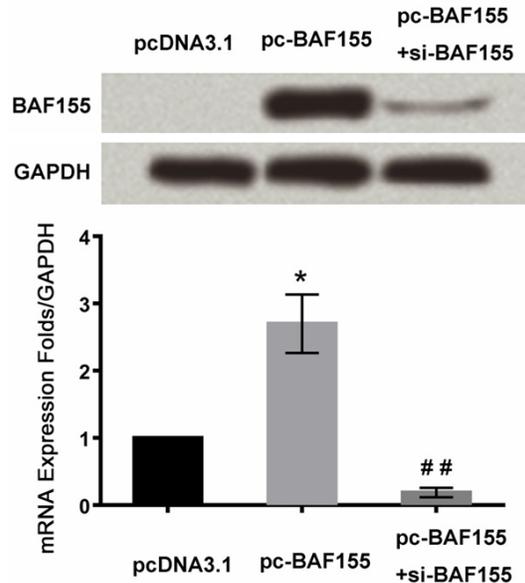


Figure 2. The expression level of BAF155 after transfection. Cells were respectively transfected with pcDNA3.1, pc-BAF155 and pc-BAF155 + si-BAF155. The expression of BAF155 was determined by Western blot analysis. Data present as mean \pm SD. *P < 0.05; ##P < 0.01. BAF, BRG1-associated factor; si-BAF155, small interfering RNA against BAF155. *indicates significant difference compared with control group; #indicates significant difference compared with cells transfected with pc-BAF155 alone.

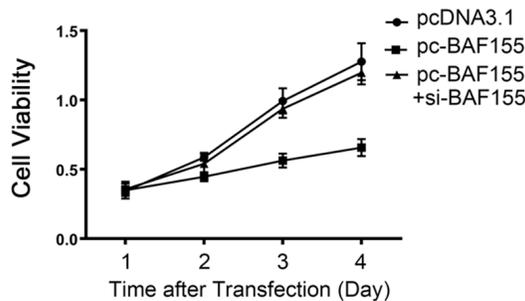


Figure 3. Over-expression of exogenous BAF155 inhibited the PC3 cell proliferation while the PC3 cell proliferation was restored to the normal level by knockdown of BAF155. The cell viability was determined by MTT assay. BAF, BRG1-associated factor; si-BAF155, small interfering RNA against BAF155.

transfected with pc-BAF155 and si-BAF155 was significantly decreased compared with that in cells transfected with pc-BAF155 alone (P < 0.05).

BAF155 inhibits PC3 cell migration

As shown in **Figure 5**, over-expressed BAF155 significantly inhibited PC3 cell migration com-

pared with that in control group (P < 0.05). However, the increased cell migration was reversed by additional transfection of si-BAF155, resulting in no obvious difference compared with control group.

BAF155 over-expression up-regulated the expression level of p16 while down-regulating the expression levels of key kinases involving in PI3K/AKT and Wnt/ β -catenin pathways

As shown in **Figure 6**, the p16 expression of cells transfected with pc-BAF155 was significantly increased compared with that in control group (P < 0.05). Over-expressed BAF155 could significantly decrease the expression levels of p/t-PI3K, p/t-AKT, Wnt3a, β -catenin and Wnt5a when compared to control group (P < 0.05). However, these proteins were restored to the normal level by additional transfection of si-BAF155.

Discussion

The SWI/SNF complex is one of several chromatin-remodeling complexes and consists of BRG1 (SMARCA4)/BRM (SMARCA2), INI1 (SMARCB1, BAF47, SNF5), BAF155 (SMARCC1), BAF170 (SMARCC2), BAF180 (PBRM1), and BAF250A (ARID1A) subunits [20, 21]. The SWI/SNF complex is critical for growth control and cancer development, and complete loss of a SWI/SNF subunit can promote cancer formation. Loss of INI1 expression has been described in pediatric renal and extrarenal malignant rhabdoid tumors, atypical teratoid/rhabdoid tumors of the central nervous system, epithelioid sarcoma and renal medullary carcinoma [22, 23]. More recently, mutations of BAF180 were identified accounting for 41% of renal cell carcinomas (RCCs), making BAF180 the second most common cancer gene in clear cell RCCs. The ARID1A subunit of SWI/SNF complexes was recently found to be mutated specifically in 50% of ovarian clear cell carcinomas and 30% of endometrioid carcinomas [24, 25]. A recent research demonstrated that ARID1A expression at both protein and mRNA levels was decreased and was also found to be correlated with high tumor stage and nuclear grade in clear cell RCC [26]. BRG1 mutations and expression loss have been found in primary lung cancers [27]. BRM has been found to be inactivated in 10-20% of solid tumor types [26, 28-31], including breast, lung, colon, bladder, ovarian, prostate, gastric, renal epithelial and

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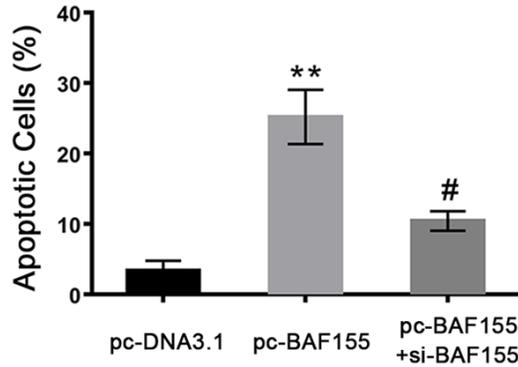


Figure 4. Over-expression of exogenous BAF155 increased the apoptosis of PC3 cells while the increase was reversed by knockdown of BAF155. The cell apoptosis was determined by flow cytometer. Data present as mean \pm SD. *P < 0.05; ##P < 0.01. BAF, BRG1-associated factor; si-BAF155, small interfering RNA against BAF155. *indicates significant difference compared with control group; #indicates significant difference compared with cells transfected with pc-BAF155 alone.

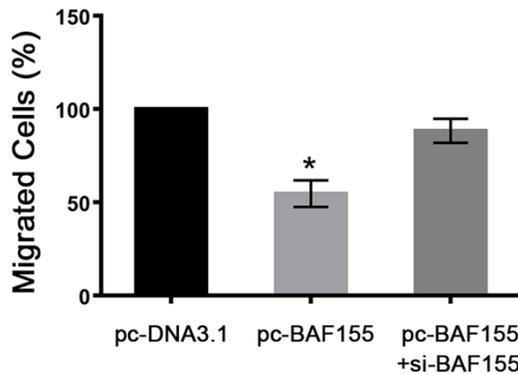


Figure 5. Over-expression of exogenous BAF155 inhibited the PC3 cell migration while PC3 cell migration was restored to the normal level by knockdown of BAF155. The cell migration was determined by the migration assay. Data present as mean \pm SD. *P < 0.05. BAF, BRG1-associated factor; si-BAF155, small interfering RNA against BAF155.

head tumors, suggesting that BRM is involved in human cancer. Moreover, BAF155, a key SWI/SNF complex subunit, has been found to lost expression in SNUC2B and SKOV3 cells [17]. In the present study, the expressions of BAF155 in PC3 and SNUC2B cells were also lost.

In the present study, we demonstrated that exogenous BAF155 could inhibit the proliferation and migration of PC3 cells while promoting the apoptosis of PC3 cells. The results indicat-

ed that exogenous BAF155 led to substantial inhibition of colony forming ability. These data showed a vital role of BAF155 in the control of neoplastic PC3 cells. These results consisted with DelBove et al [17], which attributed the pivotal role of BAF155 in cancer control to the proline/glutamine rich domain or leucine zipper of BAF155, since the transfection with $\Delta 2$ mutant lacking these domains could inactivated its ability to suppress growth in the neoplastic cell lines.

BAF155, the third core member of the SWI/SNF complex, possesses tumor suppressive capabilities related to cell cycle control. All core members of SWI/SNF complex are required for the basic task of remodeling nucleosomes, but the necessity for their presence in specific tumor suppressive functions varies. A recent research demonstrated that increased expression of BAF170 could substitute for the loss of BAF155 [32]. However, re-expression of any one of the core members could induce growth arrest via senescence, a key characteristic of many tumor suppressors.

First detected in 1994, p16 was closely associated with tumorigenesis of multiple tumors, thus is also called multiple tumor suppressor gene 1 (MTS1). p16 is a strong suppressive factor of CDK4 and plays negative feedback regulation in G1 phase. The function loss of p16 could lead to excessive proliferation, which contributes to tumorigenesis and progression of tumor. p16 gene acts as a tumor suppressor gene in many kinds of carcinomas. Down-regulation of p16 caused by gene aberrations such as gene mutation, deletion and promoter methylation, is related to carcinogenesis. It has been shown that low expression of p16 protein caused by mutation or loss of heterozygosity probably involves in tumorigenesis of primary lung carcinoma [33]. In the present study, we found that over-expression of exogenous BAF155 could up-regulate the expression of p16 in PC3 cells, indicating that BAF155 could inhibit the proliferation of PC3 cells.

Diverse processes are regulated by PI3K/Akt signaling pathway, including growth, survival and cell-cell communication. The PI3K pathway is an important intracellular mediator of survival signals stemming from trophic factors [34, 35]. PI3K is recruited and activated by cell surface receptors for peptide growth factors and

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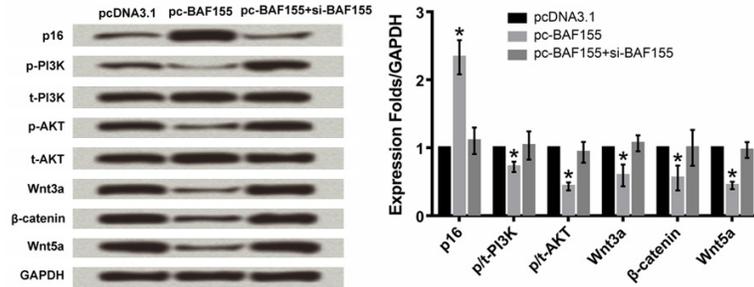


Figure 6. BAF155 over-expression up-regulated the expression level of p16 while down-regulating the expression levels of key kinases involving in PI3K/AKT and Wnt/ β -catenin pathways. The signal pathway related protein was determined by Western blot analysis. Data present as mean \pm SD. * $P < 0.05$. BAF, BRG1-associated factor; si-BAF155, small interfering RNA against BAF155; PI3K, phosphatidylinositol-3-kinase; p-PI3K, phosphorylated PI3K; p-AKT, phosphorylated AKT.

cytokines thus making exogenous peptide growth factors can directly activate intracellular cell survival pathways. Abnormal PI3K/Akt activity has been linked to the formation of tumors and metastasis including breast cancer [36], leukemia [37], melanomas [38] and lung cancer [39]. It has been shown that the PI3K/Akt pathway is a dominant growth factor-activated cell survival pathway in human LNCaP cells [40]. In the present study, we found over-expression of exogenous BAF155 could down-regulate PI3K pathway thus inhibiting PC3 cell proliferation.

Wnt signaling pathway is highly conservative in biological evolution. It not only determines the cell growth and differentiation, but also regulating the differentiation of important organs such as cardiovascular and central nervous. Wnt signal pathway directly controls the cell polarization, proliferation, differentiation and apoptosis. The disorder of Wnt signaling pathway has been linked to a variety of human tumors including esophagus cancer [41], colorectal cancer [42], breast cancer [43], and hepatocellular [44]. Accumulation of Intracellular protein β -catenin leads to Wnt signaling disorder. Meantime, free β -catenin can enter the nucleus and regulates downstream gene expression, resulting in promotion of tumorigenesis. In the present study, we found that the effect of BAF155 on Wnt signaling pathway was consistent to the results of PI3K/Akt pathway.

This paper has preliminarily studied the mechanism for inhibiting the proliferation and migration of PC3 cells by BAF155. In addition, the

cancer suppressive ability of BAF155 was further confirmed. These results will provide the theory basis for the clinical treatment of CaP.

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Disclosure of conflict of interest

None.

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References

- [1] Wang W, Xue Y, Zhou S, Kuo A, Cairns BR and Crabtree GR. Diversity and specialization of mammalian SWI/SNF complexes. *Genes Dev* 1996; 10: 2117-2130.
- [2] Sudarsanam P, Iyer VR, Brown PO and Winston F. Whole-genome expression analysis of *snf/swi* mutants of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 2000; 97: 3364-3369.
- [3] Bultman S, Gebuhr T, Yee D, Mantia CL, Nicholson J, Gilliam A, Randazzo F, Metzger D, Chambon P and Crabtree G. A *Brg1* null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol Cell* 2000; 6: 1287-1295.
- [4] Kim JK, Huh SO, Choi H, Lee KS, Shin D, Lee C, Nam JS, Kim H, Chung H, Lee HW, Park SD, Seong RH. *Srg3*, a mouse homolog of yeast SWI3, is essential for early embryogenesis and involved in brain development. *Mol Cell Bio* 2001; 21: 7787-7795.
- [5] Côté J, Quinn J, Workman JL and Peterson CL. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* 1994; 265: 53-60.
- [6] Imbalzano AN, Kwon H, Green MR and Kingston RE. Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature* 1994; 370: 481-485.
- [7] Schnitzler G, Sif S and Kingston RE. Human SWI/SNF interconverts a nucleosome between

Roles of BAF155 in PC3 cells

- its base state and a stable remodeled state. *Cell* 1998; 94: 17-27.
- [8] Shanahan F, Seghezzi W, Parry D, Mahony D and Lees E. Cyclin E associates with BAF155 and BRG1, components of the mammalian SWI-SNF complex, and alters the ability of BRG1 to induce growth arrest. *Mol Cell Biol* 1999; 19: 1460-1469.
- [9] Aravind L and Iyer LM. The SWIRM domain: a conserved module found in chromosomal proteins points to novel chromatin-modifying activities. *Genome Biol* 2002; 3: 129-137.
- [10] Aasland R, Stewart AF and Gibson T. The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIB. *Trends Biochem Sci* 1996; 21: 87-88.
- [11] Boyer LA, Latek RR and Peterson CL. The SANT domain: a unique histone-tail-binding module? *Nat Rev Mol Cell Biol* 2004; 5: 158-163.
- [12] Maitra A, Wistuba II, Washington C, Virmani AK, Ashfaq R, Milchgrub S, Gazdar AF and Minna JD. High-resolution chromosome 3p allelotyping of breast carcinomas and precursor lesions demonstrates frequent loss of heterozygosity and a discontinuous pattern of allele loss. *Am J Pathol* 2001; 159: 119-130.
- [13] Andersen CL, Christensen LL, Thorsen K, Schepeler T, Sørensen FB, Verspaget HW, Simon R, Kruhøffer M, Aaltonen LA and Laurberg S. Dysregulation of the transcription factors SOX4, CBFβ and SMARCC1 correlates with outcome of colorectal cancer. *Br J Cancer* 2009; 100: 511-523.
- [14] Heebøll S, Borre M, Ottosen PD, Andersen CL, Mansilla F, Dyrskjøt L, Orntoft TF and Tørring N. SMARCC1 expression is upregulated in prostate cancer and positively correlated with tumour recurrence and dedifferentiation. *Histol Histopathol* 2008; 23: 1069-1076.
- [15] Ralhan R, Desouza LV, Matta A, Chandra TS, Ghanny S, Datta GS, Bahadur S and Siu KW. Discovery and verification of head-and-neck cancer biomarkers by differential protein expression analysis using iTRAQ labeling, multidimensional liquid chromatography, and tandem mass spectrometry. *Mol Cell Proteomics* 2008; 7: 1162-1173.
- [16] Shadeo A, Chari R, Lonergan KM, Pusic A, Miller D, Ehlen T, Niekerk DV, Maticic J, Richards-Kortum R and Follen M. Up regulation in gene expression of chromatin remodeling factors in cervical intraepithelial neoplasia. *Bmc Genomics* 2008; 9: 1-14.
- [17] Delbove J, Rosson G, Strobeck M, Chen J, Archer TK and Wang W. Identification of a core member of the SWI/SNF complex, BAF155/SMARCC1, as a human tumor suppressor gene. *Epigenetics* 2011; 6: 1444-1453.
- [18] Appierto V, Villani MG, Cavadini E, Gariboldi M, De Cecco L, Pierotti MA, Lambert JR, Reid J, Tiberio P, Colombo N, Formelli F. Analysis of gene expression identifies PLAB as a mediator of the apoptotic activity of fenretinide in human ovarian cancer cells. *Oncogene* 2007; 26: 3952-3962.
- [19] Wang R, Wang ZX, Yang JS, Pan X, De W and Chen LB. MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). *Oncogene* 2011; 30: 2644-2658.
- [20] Reisman D, Glaros S and Thompson EA. The SWI/SNF complex and cancer. *Oncogene* 2009; 28: 1653-1668.
- [21] Wilson BG and Roberts CW. SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 2011; 11: 481-492.
- [22] Shain AH and Pollack JR. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. *PLoS One* 2013; 8: e55119.
- [23] Kadoch C, Hargreaves DC, Hodges C, Elias L, Ho L, Ranish J and Crabtree GR. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat Genet* 2013; 45: 592-601.
- [24] Bosse T, ter Haar NT, Seeber LM, v Diest PJ, Hes FJ, Vasen HF, Nout RA, Creutzberg CL, Morreau H, Smit VT. Loss of ARID1A expression and its relationship with PI3K-Akt pathway alterations, TP53 and microsatellite instability in endometrial cancer. *Mod Pathol* 2013; 26: 1525-1535.
- [25] Fadare O, Gwin K, Desouki MM, Crispens MA, Khabele D, Liang SX, Zheng W, Mohammed K and Hecht JL. The clinicopathologic significance of p53 and BAF-250a (ARID1A) expression in clear cell carcinoma of the endometrium. *Mod Pathol* 2013; 26: 1101-1110.
- [26] Lichner Z, Scorilas A, White NM, Girgis AH, Rotstein L, Wiegand KC, Latif A, Chow C, Huntsman D, Yousef GM. The chromatin remodeling gene ARID1A is a new prognostic marker in clear cell renal cell carcinoma. *Am J Pathol* 2013; 182: 1163-1170.
- [27] Glaros S, Cirrincione G, Palanca A, Metzger D and Reisman D. Targeted knockout of BRG1 potentiates lung cancer development. *Cancer Res* 2008; 68: 3689-3696.
- [28] Katagiri A, Nakayama K, Rahman MT, Rahman M, Katagiri H, Nakayama N, Ishikawa M, Ishibashi T, Iida K and Kobayashi H. Loss of ARID1A expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. *Mod Pathol* 2012; 25: 282-288.
- [29] Liu G, Gramling S, Munoz D, Cheng D, Azad AK, Mirshams M, Chen Z, Xu W, Roberts H and

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- Shepherd FA. Two novel BRM insertion promoter sequence variants are associated with loss of BRM expression and lung cancer risk. *Oncogene* 2011; 30: 3295-3304.
- [30] Shen H, Powers N, Saini N, Comstock C, Sharma A, Weaver K, Revelo M, Gerald W, Williams E and Jessen W. The SWI/SNF ATPase Brm is a gatekeeper of proliferative control in prostate cancer. *Cancer Res* 2008; 68: 10154-10162.
- [31] Yamamichi N, Inada K, Ichinose M, Yamamichinishina M, Mizutani T, Watanabe H, Shioyama K, Fujishiro M, Okazaki T and Yahagi N. Frequent loss of Brm expression in gastric cancer correlates with histologic features and differentiation state. *Cancer Res* 2007; 67: 10727-10735.
- [32] Yan Z, Wang Z, Sharova L, Sharov AA, Ling C, Piao Y, Aiba K, Matoba R, Wang W and Ko MS. BAF250B-associated SWI/SNF chromatin-remodeling complex is required to maintain undifferentiated mouse embryonic stem cells. *Stem Cells* 2008; 26: 1155-1165.
- [33] Tam KW, Zhang W, Soh J, Stastny V, Chen M, Sun H, Thu K, Rios JJ, Yang C and Marconett CN. CDKN2A/p16 inactivation mechanisms and their relationship to smoke exposure and molecular features in non-small-cell lung cancer. *J Thorac Oncol* 2013; 8: 1378-1388.
- [34] Crowder RJ and Freeman RS. Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *J Neurosci* 1998; 18: 2933-2943.
- [35] Párrizas M, Saltiel AR and Leroith D. Insulin-like growth factor 1 inhibits apoptosis using the phosphatidylinositol 3'-kinase and mitogen-activated protein kinase pathways. *J Biol Chem* 1997; 272: 154-161.
- [36] Fry MJ. Phosphoinositide 3-kinase signalling in breast cancer: how big a role might it play? *Breast Cancer Res* 2000; 3: 304-312.
- [37] Martinez LM, Anel A, Monleón I, Sierra J, Pineiro A, Naval J and Alava M. Tyrosine phosphorylation of the p85 subunit of phosphatidylinositol 3-kinase correlates with high proliferation rates in sublines derived from the Jurkat leukemia. *Int J Biochem Cell Biol* 2000; 32: 435-445.
- [38] Krasilnikov M, Adler V, Fuchs SY, Zheng D, Haimovitz-Friedman A, Herlyn M and Ronai ZE. Contribution of phosphatidylinositol 3-kinase to radiation resistance in human melanoma cells. *Mol Carcinog* 1999; 24: 64-69.
- [39] Lin X, Böhle A, Dohrmann P, Leuschner I, Schulz A, Kremer B and Fändrich F. Overexpression of phosphatidylinositol 3-kinase in human lung cancer. *Langenbeck's Arch Surg* 2001; 386: 293-301.
- [40] Lin J, Adam RM, Santiestevan E, Freeman MR. The phosphatidylinositol 3'-kinase pathway is a dominant growth factor-activated cell survival pathway in LNCaP human prostate carcinoma cells. *Cancer Res* 1999; 59: 2891-2897.
- [41] Lv J, Cao XF, Ji L, Zhu B, Tao L and Wang DD. Association of Wnt1/beta-catenin with clinical pathological characteristics and prognosis of esophageal squamous cell carcinoma. *Genet Test Mol Biomarkers* 2010; 14: 363-369.
- [42] Michael B. Crosstalk between Wnt signaling and RNA processing in colorectal cancer. *J Cancer* 2013; 4: 96-103.
- [43] Katoh M. Expression and regulation of WNT1 in human cancer: up-regulation of WNT1 by beta-estradiol in MCF-7 cells. *Int J Oncol* 2003; 22: 209-212.
- [44] Zhang X, Yang Y, Liu X, Herman JG, Brock MV, Licchesi JD, Yue W, Pei X and Guo M. Epigenetic regulation of the Wnt signaling inhibitor DACT2 in human hepatocellular carcinoma. *Epigenetics* 2013; 8: 373-382.