Ubiquitin-specific protease 4 inhibits breast cancer cell growth through the upregulation of PDCD4

YANG LI1, DAQING JIANG1, QI ZHANG1, XIAOLI LIU2 and ZHENGANG CAI3

1Department of Breast Surgery, Liaoning Cancer Hospital and Institute, Shenyang, Liaoning 110042; 2Department of Interventional Radiology, The Second Affiliated Hospital of Dalian Medical University; 3Department of Breast Surgery, The First Affiliated Hospital, Dalian Medical University, Dalian, Liaoning 116023, P.R. China

Received October 4, 2015; Accepted July 4, 2016

DOI: 10.3892/ijmm.2016.2685

Abstract. Breast cancer is a common malignant tumor affecting women. The study of the association between breast cancer and molecular aberrations may lead to the development of novel diagnostic and therapeutic strategies for the disease. In the present study, we examined the role of ubiquitin-specific protease 4 (USP4) in breast cancer. We found that USP4 expression was significantly decreased in breast cancer tissue samples compared with paired normal breast tissue samples (P<0.001). USP4 was identified as a tumor suppressor. In addition, by inducing USP4 overexpression (using a USP4 overexpression plasmid) or the knockdown of USP4 (by transfection with a USP4 shRNA plasmid), we found that USP4 inhibited cell proliferation in vitro. We also found that USP4 suppressed tumor growth by using a mouse tumor xenograft model. Moreover, programmed cell death 4 (PCD4) was identified to be a target of USP4, which plays a role as a tumor suppressor. As a whole, our findings suggest that USP4 acts as a tumor suppressor in breast cancer and that it may be an effective target for the treatment of breast cancer.

Introduction

Breast cancer is the one of most common malignancies affecting women worldwide, and is the second leading cause of cancer-related death following lung cancer (1,2). Despite the great efforts and advances that have been made in the treatment of breast cancer, the main reason for breast cancer-related deaths is metastasis (3).

As a result of gene expression assays, there is growing recognition that breast cancer is a molecularly heterogeneous disease. Breast cancer metastasis, which is responsible for almost 90% of the lethality among breast cancer patients, is a multistep process that results from alterations in genetics, including the activation of oncogenes and the inactivation and mutation of tumor suppressor genes (4,5). Though greater awareness, earlier detection, and advances in treatment, the survival of breast cancer patients has improved; however, a proportion of patients will eventually succumb to the disease as a result of metastasis (6). Therefore, there is an urgent need to identify novel genes and reveal the detailed mechanisms underlying breast cancer metastasis.

The ubiquitin-proteasome pathway is an important intracellular protein degradation regulatory system. Deubiquitination is an important opposing mechanism of ubiquitination, which is catalyzed by deubiquitinating enzymes (DUBs). DUBs can cleave ubiquitin and ubiquitin-like proteins from target proteins (7). There are approximately 100 DUBs which are encoded by human genes and are divided into 5 subclasses on account of their Ub-protease domains. The ubiquitin-specific proteases (USPs) are the largest subclass of DUBs. USP4 is a member of the USP family, which has been identified to deubiquitinate K63-linked ubiquitin conjugates from TNF receptor-associated factor (TRAF)2, TRAF6 and transforming growth factor β (TGF-β)-activated kinase 1 (TAK1) and to stabilize molecules by deubiquitinating K48-linked ubiquitination (8-11). For instance, USP4 directly interacts with and deubiquititates TGF-β type I receptor (TβR1), thereby determining the levels of TGF-β signaling (12). USP4 binds to adenosine A2A receptor and deubiquitinates it, and enhances the cell surface expression level of the receptor (13).

Programmed cell death 4 (PDCD4) suppresses neoplastic transformation by inhibiting the activation of c-Jun and consequently, AP-1-dependent transcription. It has previously been reported that PDCD4 blocks c-Jun activation by inhibiting the expression of mitogen-activated protein kinase kinase kinase 1 (MAP4K1)/hematopoietic progenitor kinase 1, a kinase upstream of Jun N-terminal kinase (JNK) (15). cDNA microarray analysis of PDCD4-overexpressing RKO human colon carcinoma cells revealed MAP4K1 as the sole target of PDCD4 on the JNK activation pathway (15). Co-transfection of a MAP4K1 promoter-reporter with PDCD4 led to the inhibition of transcription from the MAP4K1 promoter. The ectopic expression of
PDCD4 in metastatic RKO cells suppressed invasion. MAP4K1 activity is thus functionally significant in invasion, as the overexpression of a dominant-negative MAP4K1 (dnMAP4K1) mutant in RKO cells not only inhibited c-Jun activation, but also invasion (15). The overexpression of a MAP4K1 cDNA in PDCD4-transfected cells rescued the kinase activity of JNK. Thus, PDCD4 suppresses the tumor progression of human colon carcinoma cells by the novel mechanism of downregulating MAP4K1 transcription, with consequently leads to the inhibition of c-Jun activation and AP-1-dependent transcription (15).

In this study, we aimed to elucidate the function of USP4 in breast cancer. We found that USP4 expression was significantly downregulated in human breast cancer tissues. We also demonstrate that USP4 inhibits breast cancer cell proliferation. Moreover, PCD4 was identified to be a target of USP4. The present study demonstrates that USP4 may act as a potential tumor suppressor in breast cancer.

Materials and methods

Patients and tissue samples. A total of 47 breast cancer tissue samples and paired normal tissue samples were used in this study. All the samples were obtained from breast cancer patients who underwent curative resection with informed consent at the First Affiliated Hospital of Dalian Medical University, Dalian, China between 2011 and 2013. For all of the patients who participated in this study, written informed consent was obtained, and this study was approved by the Ethics Committee of Dalian Medical University. All tissues were collected immediately upon resection of the tumors in the operation theater, transported in liquid nitrogen, and then stored at -80°C.

Cells, antibodies and reagents. The normal breast epithelial cell line (MDA-MB-468) and the breast cancer cell lines (MDA-MB-468, MCF7, BT474 and BT549) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Antibodies against hyaluronic acid (HA; #5017S), urokinase plasminogen activator receptor (u-PAR; #12713S), c-Jun (#9165S), PDCD4 (#9535S), and Flag (#14793) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against MAP4K1 (#ab33910) and anti-USP4 (#ab38510) were purchased from Abcam (Cambridge, MA, USA). PDCD4 siRNA (GAACGGGAGTACCTCATT) and its control siRNA (AATGTTCCCTCAGCGACTATG) were purchased from Qiagen (Hilden, Germany). GAPDH was purchased from Abcam (Cambridge, UK) at 1:100 dilution. A semi-quantitative scoring system was used to evaluate the intensity of staining. The sections were pathologically confirmed for the tumor phenotype and specific immunostaining. The positive cells were counted and analyzed.

Western blot analysis. Proteins from the different cells and tissues were extracted in radio-immunoprecipitation assay buffer. The protein concentrations were determined using a BCA assay kit (P009; Beyotime Biotechnology, Suzhou, China). Equal amounts of protein (10-20 µg) were separated using sodium dodecyl sulfate (SDS) polyacrylamide gels and were electrotransferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). Primary antibody incubations were carried out overnight at 4°C, followed by their respective secondary antibodies. β-actin was used as a loading control. The membranes were washed 3 times with TBST and visualized with chemiluminescence (Thermo Scientific, Rockford, IL, USA).

RT-qPCR. Total RNA was isolated from the cells and tissues using TRIzol reagent, according to the manufacturer’s instructions (Invitrogen). cDNA was generated from 1 µg total RNA using SuperScript III (Invitrogen) and polyN primers. qPCR was performed using the StepOne and StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. The primer sequences were as follows: USP4 forward, 5'-AACACCTAGAGCTGTTAGC-3' and reverse, 5'-CATAGTTCAACCACAGACA-3; PDCD4 forward, 5'-AAACCCGAGAAATGCTGG-3' and reverse, 5'-TGCCAAACCTGTGACTCCAC-3' and PDCD4 forward, 5'-GC CGACCTCTTTTTTGGCTGC-3' and reverse, 5'-TCCC GTTCTCAGCTTGGAGCTG-3'. The relative levels of gene expression were represented as 2^(-ΔΔCt) (Ct gene - Ct reference). The experiments were repeated in triplicate.
In vivo tumor formation assay. The in vivo tumor formation assay was performed as previously described (14). Briefly, 1x10⁶ cells (BT549 USP4-overexpressing cells, MCF7 cells in which USP4 had been knocked down and the respective controls) were subcutaneously injected into the right flanks of severe combined immunodeficient (SCID) mice (n=12, 6 weeks old, 20±2 g; Shanghai Slac Laboratory Animal Co., Ltd., Shanghai, China). Tumor length (L) and width (W) were measured every 3 days, and the tumor volume was calculated using the following equation: volume = (W² x L)/2. The mice were housed for 25 days post-inoculation. Mice were maintained under a 12-hour light/12-hour dark cycle at 24°C with free access to water and a standard mouse diet (66% carbohydrate, 12% fat, 22% protein). All the mice were then euthanized by CO₂ inhalation and the tumor tissues were removed by surgical excision. The animal experiments were performed following the approval of the Animal Care and Use Committee of Dalian Medical University.

Coimmunoprecipitation assay. Flag-tagged PDCD4 plasmid construction was as follows: Full-length PDCD4 was cloned into a 3XFLAG-tagged CMV10 vector, which was subsequently cloned into a pBabe.puro retroviral vector (Cell Biolabs, San Diego, CA, USA). HA-tagged USP4 plasmid construction was as follows: Full-length USP4 was cloned into a HA-tagged CMV10 vector, which was subsequently cloned into a PBABE-Puro retroviral vector. The 293T cells were co-transfected with Flag-tagged PDCD4 and HA-tagged USP4 constructs. The cells were then seeded in 10-cm culture dishes and then lysed in NP-40 lysis buffer. Approximately 1 mg cell lysates were incubated with the Flag antibody overnight at 4°C on a vortical roller. The lysates were then incubated with 20 µl of Protein A agarose beads (Calbiochem) for 4 h and the beads were then washed 4 times with cold PBS. The immunoprecipitated proteins were then identified by western blot analysis. The membranes were immuno-blotted with the corresponding primary antibodies.

Gene expression profiling. Affymetrix Hu U133 plus 2.0 arrays were used according to manufacturer’s instructions. The data were initially normalized by robust multiarray average (RMA) normalization algorithms in expression console software. The genes with significant alterations in expression between the USP4-overexpressing cells and the corresponding controls were identified by scatter plots and the that were genes up- and downregulated by ≥5-fold were identified as significant. Cluster analysis was carried out using the gene list by Gene Cluster v3.0 software, and heatmaps were created using Java TreeView v1.1.4r3 software. Gene set enrichment analysis was carried out using ConceptGen.

Protein degradation assay. MG132 (carbobenzoxy-Leu-Leu-leucinal) was purchased from Sigma-Aldrich. MG132 is a peptide aldehyde, which effectively blocks the proteolytic activity of the 26S proteasome complex. We measured the PDCD4 expression level following treatment with MG132 (5 µM) in different USP4 conditions to confirm whether USP4 inhibits PDCD4 degradation.

Statistical analysis. The results were analyzed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). The differences between 2 groups were analyzed using a two-tailed t-test. The results are presented as the means ± SD. P-values <0.05 were considered to indicate statistically significant differences.

Results

Expression of USP4 is downregulated in breast cancer tissues. USP4 expression was analyzed in 47 breast cancer tissue samples compared with those in the adjacent normal tissue samples by immunohistochemistry. The human normal breast tissues exhibited greater immunostaining, whereas the breast cancer tissues exhibited less immunostaining (Fig. 1A and B). USP4 protein, which is located in the nuclei of tumor cells, was ubiquitously expressed in all human breast tissues. We also compared the expression levels of USP4 between tumor and normal breast tissues by western blot analysis. The USP4 protein levels were found to be decreased in the breast cancer tissues compared with the matched normal tissues (Fig. 1B and C), indicating a possible protective role for USP4 in the development of breast cancer.

Establishment of stable USP4 expression in breast cancer cell lines. Western blot analysis and RT-qPCR were used to measure the USP4 protein and mRNA expression levels,
USP4 inhibits breast cancer cell growth by upregulating PDCD4

respectively in the normal breast epithelial cell line (MCF10A) and in the breast cancer cell lines (MDA-MB-468, MCF7, BT474 and BT549) (Fig. 2). We used the BT549 breast cancer cell line to establish a stable cell line which overexpressed USP4 protein with the purpose of revealing the role of USP4 in the occurrence and progression of breast cancer. We also used shRNA to generate a stable cell line (the MCF7 breast cancer cells) in which USP4 was knocked down. The transfection efficiency was confirmed by western blot analysis and RT-qPCR. The BT549 cells that had been transfected with the USP4 overexpression plasmid exhibited a significantly increased expression of USP4 at both the mRNA and protein level compared with the control vector (mock)-transfected cells (Fig. 3A and B). On the contrary, the MCF7 cells that had been transfected with the USP4 shRNA plasmid exhibited a significantly decreased expression of USP4 at both the mRNA and protein level compared with the cells transfected with the control shRNA (Fig. 3C and D).

**USP4 inhibits breast cancer cell proliferation.** At first, the effects of USP4 expression on cell proliferation were examined by MTT assay. As shown in Fig. 4A, the overexpression of USP4 significantly inhibited the proliferation of BT549 cells, whereas USP4 knockdown significantly promoted the proliferation of the MCF7 cells (Fig. 4B). We then performed a human tumor clonogenic assay to confirm the effects of USP4 on cell proliferation. We found that overexpression of USP4 significantly reduced the colony-forming ability of the BT549 cells, whereas the colony-forming ability of the MCF7 cells was significantly enhanced by transfection with USP4 shRNA (Fig. 4C and D). These results indicated that USP4 markedly inhibited breast cancer cell proliferation and colony-forming ability.

In addition, in *in vivo* experiments, we created a mouse xenograft tumor model using SCID mice to explore the effects of USP4 on tumorigenesis *in vivo*. The diameters of the tumors were measured every 3 days. We found that the

---

**Figure 2.** Expression of ubiquitin-specific protease 4 (USP4) in breast cancer cells. (A) USP4 protein levels in breast cancer and normal cells were assessed by western blot analysis. (B) USP4 mRNA levels in breast cancer cells were assessed by RT-qPCR.

**Figure 3.** Establishment of ubiquitin-specific protease 4 (USP4)-overexpressing cells and cells in which USP4 was silenced. (A) The transfection efficiency of USP4 in BT549 cells was analyzed by measuring the protein levels by western blot analysis. β-actin was used as a loading control. (B) The transfection efficiency of USP4 in BT549 cells was analyzed by measuring the mRNA levels by RT-qPCR. (C) The transfection efficiency of shUSP4 in MCF7 cells was analyzed by measuring the protein levels by western blot analysis, β-actin was used as a loading control. (D) The transfection efficiency of shUSP4 in MCF7 cells was analyzed by measuring the mRNA levels by RT-qPCR. **P<0.01, based on the Student’s t-test. All results are from 3 or 4 independent experiments. Error bars indicate the means ± standard deviation.
mice that had been injected with USP4-overexpressing BT549 cells did not form tumors until the 12th day, whereas those that had been injected with the control BT549 cells formed tumors on the 9th day. The tumors in the USP4 overexpression group grew relatively slower than those in the mice that had been injected with the control cells during the subsequent days (Fig. 5A and B). Similar results were observed with the MCF7 cells. We also found that the mice injected with the cells in which USP4 was knocked down formed tumors earlier and that the tumor volumes in these mice were much larger than those that were formed from the control cells (Fig. 5C and D). These results suggest that USP4 inhibits breast cancer cell growth and proliferation in vivo.

USP4 upregulates PDCD4 expression in breast cancer cells. To better understand the mechanisms through which USP4 is involved in breast cancer cell proliferation, we performed gene expression profiling using the USP4-overexpressing BT549 cells and the respective control cells. Microarray analyses identified a list of genes that were significantly differentially expressed following the ectopic expression of USP4, including the upregulation of PDCD4 signaling (Fig. 6A). Furthermore, gene set enrichment analysis indicated that PDCD4-related gene signatures were significantly altered in the USP4-overexpressing breast cancer cells (Fig. 6B). These data also led us to hypothesize that USP4 exerts these functions possibly via PDCD4. To examine this hypothesis, we first determined whether PDCD4 is a downstream target of USP4 in breast cancer cells. The expression of PDCD4 in the cells with am altered expression of USP4 was further evaluated by western blot analysis and RT-qPCR. The results revealed that the PDCD4 protein expression levels were significantly increased by USP4 ectopic expression in the BT549 cells and were significantly decreased by USP4 knockdown in the MCF7 cells (Fig. 7A). It should be noted that the PDCD4 mRNA level exhibited no significant change in both cell lines (BT549 USP4-overexpressing cells and MCF7 cells in which USP4 was knocked down) (Fig. 7B), indicating that the regulatory effects of USP4 on PDCD4 expression only take place at the post-transcriptional level.

To elucidate the mechanisms responsible for the effects of USP4 on PDCD4, 293T cells were co-transfected with Flag-tagged PDCD4 and HA-tagged USP4 constructs. We found that HA-tagged USP4 co-immunoprecipitated with Flag-tagged PDCD4 by using immunoprecipitation and subsequent western blot analyses, confirming a physical interaction between the two proteins (Fig. 7C). The physical interaction between USP4 and PDCD4 indicated that USP4 targets PDCD4 for deubiquitination and subsequent inhibition of its degradation. Furthermore, the knockdown of USP4 significantly decreased the endogenous PDCD4 protein expression level (Fig. 7D). In addition, proteasome inhibition by the addition of MG132 induced a significant
LI et al: USP4 INHIBITS BREAST CANCER CELL GROWTH BY UPREGULATING PDCD4

increase in the PDCD4 expression level (Fig. 7D), suggesting that USP4 inhibits PDCD4 degradation.

**USP4 inhibits breast cancer cell proliferation through the upregulation of PDCD4.** PDCD4 is closely associated with proliferation, migration and invasion of various cancer types, including breast cancer (16). Thus, in this study, we wished to determine whether PDCD4 is involved in the USP4-mediated inhibition of breast cancer cell proliferation. We examined the effects of USP4 on PDCD4 expression in the BT549 and MCF7 cells by measuring the expression of u-PAR, MAP4K1 and c-Jun. As shown in Fig. 8A, the upregulation of USP4...
significantly inhibited the expression of u-PAR, MAP4K1 and c-Jun, and the knockdown of USP4 significantly upregulated the expression of u-PAR, MAP4K1 and c-Jun (Fig. 8B). To determine whether PDCD4 is involved in the anti-proliferative function of USP4, we further examined the role of PDCD4 in the USP4-induced inhibition of proliferation by knocking down PDCD4 using siRNA. The PDCD4 knockdown efficiency was confirmed by western blot analysis at 48 h post-transfection (Fig. 9A). The targets of PDCD4 (u-PAR, MAP4K1 and c-Jun) were significantly upregulated in the BT549 USP4-overexpressing cells following transfection with siPDCD4 (Fig. 9B). As shown in Fig. 9C, the inhibition of cell proliferation that was induced by USP4 overexpression was markedly reversed following PDCD4 knockdown using siRNA. These results indicate that PDCD4 is involved in the USP4-mediated inhibition of the proliferation of breast cancer cells.

Discussion

Cancer statistics indicate that there were 226,870 new breast cancer cases in 2014 in the US. Breast cancer is thought to comprise more than a quarter of all new female cancer cases. A total of 39,510 breast cancer-related deaths were estimated to have occurred, accounting for approximately 14% of all cancer-related deaths (17). In spite of the decline in breast
cancer-related deaths, a large number of breast cancer patients develop metastatic tumors even after surgical tumor resection (18,19). In the present study, we identified USP4 as a potential target gene for the inhibition of breast cancer cell proliferation and tumor growth.

USP4 is overexpressed in various types of cancer and the increased levels of USP4 appear to play a functional role in malignant cells (9,20-22). For example, USP4 promotes tumorigenesis when overexpressed in mice. USP4 interacts directly with and deubiquitinates ADP-ribosylatibon factor-binding protein 1 (ARFBP1), which results in the stabilization of ARF-BP1 and the subsequent reduction of p53 levels (11). These findings suggest an oncogenic potential for USP4. However, the effects of USP4 on oncogenesis are controversial. Some studies have shown that USP4 expression is decreased in lung cancer cell lines (23). Furthermore, USP4 has been verified as a negative regulator of the Wnt signaling pathway, which has tumorigenic activity (24). The mechanistic connection between USP4 and PDCD4 was previously unknown. In this study, we proved that USP4 altered PDCD4 expression. Our results revealed that the PDCD4 expression level was significantly increased in USP4-overexpressing cells, whereas the PDCD4 level was decreased in cells in which USP4 was knocked down. When we transfected the USP4-overexpressing cells with PDCD4 siRNA, the inhibitory effects of USP4 on cell proliferation were reversed. These data demonstrate that USP4 suppresses the proliferation of breast cancer cells through the upregulation of PDCD4. However, further studies are required to elucidate the exact mechanisms through which USP4 regulates PDCD4.

In conclusion, in this study, we found that USP4 expression was downregulated in breast cancer lesions compared with matched non-tumor tissues. Our data demonstrate that USP4 plays an important role in inhibiting breast cancer cell proliferation through the upregulation of PDCD4. Thus, we consider USP4 as a potential candidate tumor suppressor gene and USP4 may thus prove to be a novel therapeutic target in the management of breast cancer.
Acknowledgements

This study was supported by the Natural Science Foundation of China (grant no. 81172180).

References

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin 62: 10-29, 2012.
2. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
3. Steeg PS: Tumor metastasis: Mechanistic insights and clinical challenges. Nat Med 12: 895-904, 2006.
4. Byler S, Goldgar S, Heerboth S, Leary M, Housman G, Moulton K and Sarkar S: Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer Res 34: 1071-1077, 2014.
5. Sun Y, Wang Y, Fan C, Gao P, Wang X, Wei G and Wei J: Estrogen promotes stemness and invasiveness of ER-positive breast cancer cells through Gli1 activation. Mol Cancer 13: 137, 2014.
6. Geng SQ, Alexandreau AT and Li JF: Breast cancer stem cells: Multiple capacities in tumor metastasis. Cancer Lett 349: 1-7, 2014.
7. Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK and Bernards R: A genomic and functional inventory of deubiquitinating enzymes. Cell 123: 773-786, 2005.
8. Fan YH, Yu Y, Mao RF, Tan XJ, Xu GF, Zhang H, Lu XB, Fu SB and Yang J: USP4 targets TAK1 to downregulate TNFα-induced NF-κB activation. Cell Death Differ 18: 1547-1560, 2011.
9. Xiao N, Li H, Luo J, Wang R, Chen H, Chen J and Wang P: Ubiquitin-specific protease 4 (USP4) targets TRAF2 and TRAF6 for deubiquitination and inhibits TNFα-induced cancer cell migration. Biochem J 441: 979-986, 2012.
10. Zhou F, Zhang X, van Dam H, Ten Dijke P, Huang H and Zhang L: Ubiquitin-specific protease 4 mitigates Toll-like/interleukin-1 receptor signaling and regulates innate immune activation. J Biol Chem 287: 11002-11010, 2012.
11. Zhang X, Berger FG, Yang J and Lu X: USP4 inhibits p53 through deubiquitination and stabilizing ARF-BP1. EMBO J 30: 2177-2189, 2011.
12. Zhang L, Zhou P, Drabsch Y, Gao R, Snaar-Jagalska BE, Mickanin C, Huang H, Sheppard KA, Porter JA, Lu CX, et al: USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF-β type I receptor. Nat Cell Biol 14: 717-726, 2012.
13. Milojnovic T, Reiterer V, Stefan E, Korkhov VM, Dorostkar MM, Duzca E, Ogris E, Boehm S, Freissmuth M and Nanoff C: The ubiquitin-specific protease Usp4 regulates the cell surface level of the A2A receptor. Mol Pharmacol 69: 1083-1094, 2006.
14. Wang Y, Wen M, Kwon Y, Xu Y, Liu Y, Zhang P, He X, Wang Q, Huang J, et al: CUL4A induces epithelial-mesenchymal transition and promotes cancer metastasis by regulating ZEB1 expression. Cancer Res 74: 520-531, 2014.
15. Yang HS, Matthews CP, Clair T, Wang Q, Baker AR, Li CC, Tan TH and Colburn NH: Tumorigenesis suppressor Pdc4d down-regulates mitogen-activated protein kinase kinase kinase lsexpression to suppress colon carcinoma cell invasion. Mol Cell Biol 26: 1297-1306, 2006.
16. Chen Z, Yuan YC, Wang Y, Liu Z, Chan HJ and Chen S: Down-regulation of programmed cell death 4 (PDCD4) is associated with aromatase inhibitor resistance and a poor prognosis in estrogen receptor-positive breast cancer. Breast Cancer Res Treat 152: 29-39, 2015.
17. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014.
18. Luo M, Brooks M and Whic M: Epithelial-mesenchymal plasticity of breast cancer stem cells: implications for metastasis and therapeutic resistance. Curr Pharm Des 21: 1301-1310, 2015.
19. Raap M, Antonopoulos W, Dämmlrich M, Christgen H, Steinmann D, Länger F, Lehmann U, Kreipe H and Christgen M: High frequency of lobular breast cancer in distant metastases to the orbit. Cancer Med 4: 104-111, 2015.
20. Hou X, Wang L, Zhang L, Pan X and Zhao W: Ubiquitin-specific protease 4 promotes TNF-α-induced apoptosis by deubiquitination of RIP1 in head and neck squamous cell carcinoma. FEBS Lett 587: 311-316, 2013.
21. Uras IZ, List T and Nijman SM: Ubiquitin-specific protease 4 inhibits mono-ubiquitination of the master growth factor signaling kinase PDK1. PLoS One 7: e31003, 2012.
22. Luna-Vargas MP, Faesen AC, van Dijk WJ, Raper M, Fish A and Sixma TK: Ubiquitin-specific protease 4 is inhibited by its ubiquitin-like domain. EMBO Rep 12: 365-372, 2011.
23. Frederick A, Rolfe M and Chi M: The human UNP locus at 3p21.3 encodes two tissue-selective, cytoplasmic isofoms with deubiquitinating activity that have reduced expression in small cell lung carcinoma cell lines. Oncogene 16: 153-165, 1998.
24. Zhao B, Schliesiger C, Masucci MG and Lindsten K: The ubiquitin specific protease 4 (USP4) is a new player in the Wnt signalling pathway. J Cell Mol Med 13: 1886-1895, 2009.
25. Frederdersdorf S, Burns J, Milne AM, Packham G, Fallis L, Gillette CE, Royds JA, Peston D, Hall PA, Hanby AM, et al: High level expression of p27(kipl) and cyclin D in some human breast cancer cells: Inverse correlation between the expression of p27(kipl) and degree of malignancy in human breast and colorectal cancers. Proc Natl Acad Sci USA 94: 6380-6385, 1997.
26. Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A, Pawitan Y, Hall P, Klaar S, Liu ET, et al: An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc Natl Acad Sci USA 102: 13550-13555, 2005.
27. Mills GB, Lu Y, Fang X, Wang H, Eder A, Mao M, Sway R, Cheng KW, Stokoe D, Siminovitch K, et al: The role of genetic abnormalities of PTEN and the phosphatidylinositol 3-kinase pathway in breast and ovarian tumorigenesis, prognosis, and therapy. Semin Oncol 28 (Suppl 16): 125-141, 2001.
28. Xu Y, Wang Y, Ma G, Wang Q and Wei G: CUL4A is overexpressed in human pituitary adenomas and regulates pituitary tumor cell proliferation. J Neurooncol 116: 625-632, 2014.
29. Santhanam AN, Baker AR, Hegamyer G, Kirschmann DA and Colburn NH: Pdc4d repression of lysyl oxidase inhibits hypoxia-induced breast cancer cell invasion. Oncogene 29: 3921-3932, 2010.
30. Fischer N, Gøke F, Splitsstösser V, Lankat-Buttgereit B, Müller SC and Ellinger J: Expression of programmed cell death protein 4 (PDCD4) and miR-21 in urothelial carcinoma. Biochem Biophys Res Commun 417: 29-34, 2012.
31. Allgayer H: Pdc4d, a colon cancer prognostic that is regulated by a microRNA. Crit Rev Oncol Hematol 73: 185-191, 2010.