

ID1 Controls Aggressiveness of Salivary Gland Cancer Cells *via* Crosstalk of IGF and AKT Pathways

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Abstract. *Background: Inhibitor of differentiation or DNA binding 1 (ID1) is overexpressed in human salivary gland cancer (SGC). The insulin growth factor (IGF) system is an attractive target in cancer control because it is associated with various cancer progressions. Materials and Methods: The human SGC cell line HSY with abundant ID1 was used. ID1 knockdown and its effect on the IGF system were investigated. Cell proliferation and invasion, as well as associated protein expression, were analyzed. Phospho-AKT was also evaluated. Results: ID1 knockdown reduced cell proliferation and invasion, while the expression of proteins associated with malignant phenotypes was altered. IGF-II expression was suppressed, suggesting that this system is one of the mechanisms underlying effects of ID1 in SGC cells. c-Myc was up-regulated, whereas p21 and p27 were down-regulated. Moreover, phospho-AKT was reduced in ID1-knockdown cells. Conclusion: ID1 down-regulation induced parallel changes in the IGF and AKT pathways. The crosstalk of these pathways may enhance malignant phenotypes in SGCs.*

Growth factors secreted by cancer cells can drastically modify the tumor microenvironment (1, 2). The insulin-like growth factor (IGF) system is one of the attractive targets for cancer therapy (3, 4). Elevated expression of the IGF-I receptor (IGF-IR) and its ligands, IGF-I and IGF-II, has been reported in human cancers (5, 6). IGFs bind to two receptor

tyrosine kinases, the IGF-1R and the insulin receptor (IR), as well as their hybrid receptors (7).

We previously reported that the ectopic expression of inhibitor of differentiation or DNA binding (ID1) leads to increased proliferation, invasion and metastasis of breast and salivary gland cancer (SGC) cells (8, 9). Moreover, cancer cell malignant phenotypes are also controlled by sex steroid hormones and their receptor systems (10, 11). This phenomenon results from the fact that *ID1* presents a sex steroid hormone-responsive element in its promoter (12). Therefore, this hormonal inhibition of malignant phenotypes might be specific to ID1 regulation. The IGF system is involved in organ development (13). ID protein family is also strongly associated with organ development. Only few studies reported the relationship between ID1 and the IGF system (14, 15). One report mentioned that ID1 can increase IGF-II expression (15). Thus, the relationship between ID1 and the IGF system should be investigated further.

Additionally, a variety of growth factors and cytokines exert their effects on cancer cells by activating the AKT signaling pathway that regulates various malignant phenotypes in cancer cells, including proliferation, survival, angiogenesis, invasion and metastasis (16). Herein, we showed that ID1, IGF-II and the AKT pathway crosstalk are involved in the maintenance of SGC cell proliferation and invasion.

Materials and Methods

Cell culture. To evaluate the ID1 expression level, three cell lines derived from human SGC were screened. HSG and HSY cells were established from human SGC (17, 18). These cell lines were a generous gift from Professor Sato (Tokushima University, Tokushima, Japan). ACC2 cells, also derived from human SGC, were purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Science (Shanghai, China). Briefly, cells were cultured in Roswell Park Memorial Institute 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 5% fetal bovine serum at 37°C in an atmosphere of 5% CO₂.

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Transfection of the ID1-specific shRNA and control vector. For the specific expression, the ID1-shRNA coding sequence carried in the pGIPZ expression vector (Thermo Scientific, Waltham, MA, USA) and the control empty vector were transfected into HSY cells using Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA). The ID2 protein contains a helix-loop-helix (HLH) motif similar to that of ID1 (90% identity) (19). Cells were seeded and selected in 0.6 mg/ml puromycin. We obtained cell lines that contained each vector from the transfected HSY cells. HSY-ID1AS cells and controls were used for further experiments, after screening of the expression level by Western blotting. The cell lines were compared with the original cells in the experiments for each transfectant.

Northern blot analysis and reverse transcription-polymerase chain reaction ((RT)-PCR). Total cellular RNA was isolated and purified. RNA (15 µg) was separated by electrophoresis through formaldehyde-agarose gels and transferred to a nylon membrane (Hybond N; Amersham Biosciences, Piscataway, NJ, USA). The membrane was hybridized with a ³²P-labeled human ID1-cDNA probe (20) (a kind gift from Dr. Hartmut Land (ICRF, London, UK)), washed and exposed to XAR-5 film (Kodak, Tokyo, Japan) for autoradiography. The MMP9 probe was generated by RT-PCR using the following primer set: 5'-CACTGTCCACCCCTCAG AGC-3' and 5'-GCCACTTGTCGGCGATAAGG-3'. Ribosomal 28S and 18S RNA were used as control for RNA integrity and quantity.

Western blot analysis. Cells were collected and lysed in 2× Laemmli buffer and stored at -70°C. Protein concentration was determined using the DC Protein Assay kit (Bio-Rad, Hercules, CA, USA). For IGF-II, Bio-Plex kit (Bio-Rad) was used to collect the proteins from the secretory fraction. Samples (20-30 µg of total protein) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Hybond P; Amersham Biosciences, Little Chalfont, UK). Membranes were blocked for 1 h at room temperature with Tris-buffered saline with Tween-20 (20 mM Tris, 137 mM NaCl, 3.8 mM HCl and 0.1% Tween-20) containing 5% nonfat milk and blots were probed with anti-ID1, (Z-8; Santa Cruz Biotechnology, Santa Cruz, CA, USA) anti-IGF-IR (ab80548; Abcam plc, Cambridge, UK), anti-p21 (ab26955; Abcam plc), anti-p27 (ab37705; Abcam plc), anti-c-myc (9E10; Santa Cruz Biotechnology), anti-IGF-II (R&D Systems, Minneapolis, MN, USA), anti-AKT (610860; BD Biosciences Pharmingen, San Diego, CA, USA), anti-pAKT (Thr308) (558275; BD Biosciences Pharmingen), anti-p-AKT (Ser473) (560404; BD Biosciences Pharmingen), anti-MMP9 (ab35326; Abcam plc) or anti-Actin (C4; Chemicon, Temecula, CA, USA) antibodies for 1 h. Membranes were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Santa Cruz Biotechnology), washed and developed by enhanced chemiluminescence using the Amersham ECL-Plus kit according to the manufacturer's instructions.

MTT assay. To quantify cell proliferation, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrasodium bromide (MTT) assay was used (Chemicon). Cells were seeded and checked in various densities. As a result, 3×10³ cells/well were seeded for the 72 h experiments to obtain optimal cell density in 96-well plates. Upon completion of treatments, cells were incubated at 37°C with

MTT for 4 h and, then, isopropanol with 0.04 M HCl was added and the absorbance was read after 1 h in a plate reader at a test wavelength of 570 nm. The absorbance of the media alone at 570 nm was subtracted and % control was calculated as the absorbance of the treated cells/control cells ×100 (%).

Boyden chamber invasion assay. Assays were performed in modified Boyden chambers with 8-µm-pore filter inserts for 24-well plates (Collaborative Research, Bedford, MA, USA). Filters were coated with 12 µl of ice-cold Matrigel (11 mg/ml protein; Collaborative Research). HSY-ctl and HSY-IDAS cells (40,000 cells/well) were added to the upper chamber in 200 µl of serum-free medium. Cells were assayed in triplicate or quadruplicate and the results were averaged. The lower chamber was filled with 300 µl of conditioned medium from fibroblasts. After 20 h of incubation, cells were fixed with 2.5% glutaraldehyde in PBS and stained with 0.5% toluidine blue in 2% Na₂CO₃. Cells that remained in the Matrigel or were attached to the upper side of the filter were removed with cotton swabs. Cells on the lower side of the filter were counted using light microscopy.

Zymography. Proliferating HSY cells (1×10⁶ cells in 100-mm dishes) were placed in serum-free medium for 2-3 days, at which time the medium was replaced by 10 ml of fresh serum-free medium. After 48 h, the conditioned medium was collected and concentrated 10- to 15-fold using 10 kDa cut-off filters (Millipore, Bedford, MA, USA). The concentrated medium was analyzed on gelatin substrate gels, as described by Fisher and Werb (21). Briefly, gels consisted of 8-10% polyacrylamide and 3 mg/ml gelatin (Sigma). Concentrated conditioned medium was mixed with non-reducing Laemmli sample buffer and incubated at 37°C for 15 min. After electrophoresis, the gels were incubated for 1 h in 2.5% Triton X-100 at room temperature followed by 24-48 h in substrate buffer (100 mM Tris-HCl (pH 7.4) and 15 mM CaCl₂). The gels were stained with Coomassie Blue for 30 min and destained with 30% methanol/10% acetic acid.

Statistical analysis. Statistical comparisons were performed using the two-tailed student *t*-test. *p*<0.05 was regarded as significant. SPSS version 22.0 (IBM, Armonk, NY, USA) was used for analyses.

Results

Endogenous mRNA and protein expression of ID1 and IGF-IR. ID1 protein expression was analyzed by western blotting in the three cell lines. After screening of the expression levels of ID1 in three SGC cell lines, HSY was selected for further experiments because it showed high expression of ID1. A positive correlation was observed between the mRNA and protein expression levels in the HSY cell lines. IGF-IR was also expressed in the HSY cell lines (Figure 1A and B).

Introduction of ID1-specific shRNA in HSY cells and its influence on IGF-II expression. As described in Materials and Methods, HSY cells were transfected with the empty and ID1AS-vector. As controls (ctl cells), we used cells transfected with the pGIPZ empty vectors (HSY-ctl), which

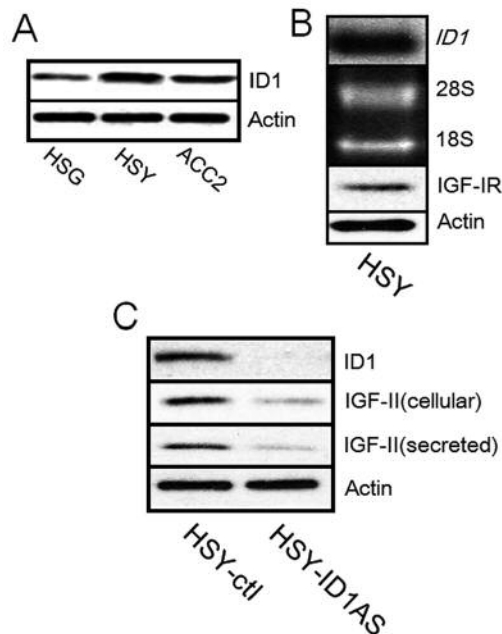


Figure 1. Inhibitor of differentiation 1 (ID1) expression profiles in salivary gland cells. A. ID1 protein expression was analyzed by Western blotting in three cell lines. B. In HSY cells, ID1 mRNA expression was analyzed by Northern blotting and IGF-IR expression was analyzed by Western blotting. C. ID1 knockdown in HSY cells. Expression of IGF-II in both the cellular and secretory fractions decreased as ID1 was knocked-down.

expressed ID1 at the same level as the original cell lines (Figure 1B and C).

ID1 knockdown resulted in a decrease in IGF-II protein levels in both the cellular and secretory fractions.

Cell proliferation of HSY-ctl and HSY-ID1AS cells after transfection. ID1AS transfection drastically reduced cell proliferation (Figure 2A). Compared to ctl cells, cell proliferation was significantly suppressed in HSY-ID1AS cells.

Moreover, the cell morphology was modified in HSY-ID1AS cells compared with that in ctl cells (Figure 2B); the cells were flattened and could not form a monolayer in confluent conditions.

Expression of ID1, AKT, phospho-AKT and other proteins. The expression of various proteins was determined after ID1 knockdown. ID1 knockdown resulted in a decrease in c-myc expression, whereas p21 and p27 were up-regulated (Figure 3A). Additionally, western blotting indicated that the quantity of p-AKT (Thr308 and Ser473) was reduced in HSY cells with ID1 knockdown (Figure 3B).

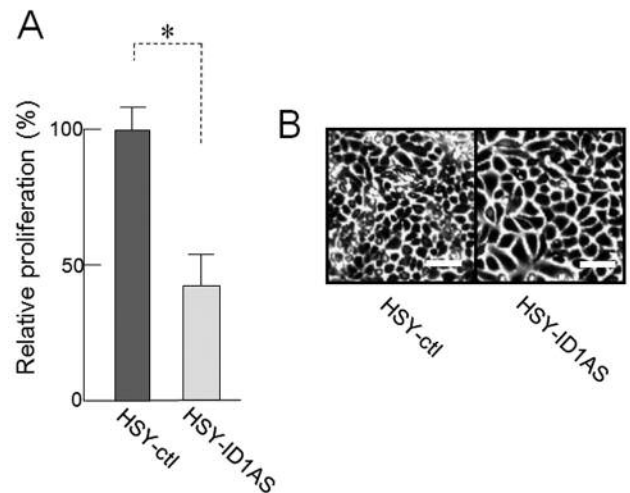


Figure 2. Cell proliferation and morphology. A. Cell numbers after ID1AS transfection were significantly reduced. B. Morphological changes of HSY-ID1AS-transfected cells. Cells cultured in 5% serum were photographed when they were confluent. Scale bar=150 μ m.

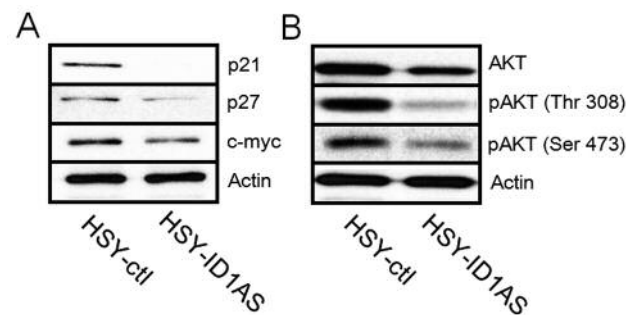


Figure 3. Cell cycle-related protein expression and AKT phosphorylation after ID1 knockdown. A. Expression of various proteins after ID1 knockdown. c-myc Expression was similar to that of ID1, whereas p21 and p27 were up-regulated. B. Involvement of the AKT pathway in ID1-associated SGC cell proliferation. Western blotting indicates that the quantity of pAKT (Thr308, Ser473) was reduced in SGC cells with ID1 knockdown.

Boyden chamber invasion assay and zymography. ID1 knockdown significantly reduced the invasion activity of HSY cells (Figure 4A). Gelatin zymographic analysis of MMP9 gelatinase secreted by HSY-ctl cells and by HSY-ID1AS cells was performed. Zymography, western and Northern blotting analyses indicated that MMP9 was drastically suppressed by ID1 knockdown (Figure 4B).

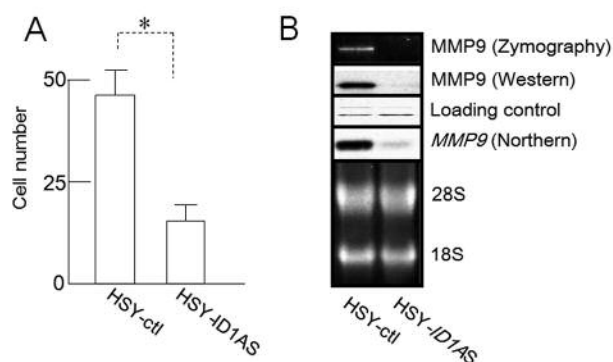


Figure 4. Cell invasiveness. A. Matrigel invasion assay shows that *ID1* knockdown inhibited cell invasion. B. Gelatin zymographic analysis of MMP9 gelatinase secreted by HSY-ctl cells and HSY-ID1AS cells. Zymography, western and Northern blotting analyses were performed as described in the Materials and Methods.

Discussion

IGF-II is commonly expressed by tumor cells and may act as an autocrine growth factor to reach target tissues (22). The IGF-IR is commonly overexpressed in many cancers and many studies have identified new signaling pathways involving the IGF-IR that affect cancer cell proliferation, adhesion and migration (22). *ID1* knockdown has been shown to reduce IGF-II expression in the intracellular and secretory fractions. Additionally, inhibition of IGF-II production was previously shown to impair the transcriptional expression of basic HLH proteins, such as *MyoD* (23). *ID1*, thus, affects the transcription of *MyoD*. Taken together, these data led us to investigate the association between the *ID1* and IGF systems.

As expected, *ID1* knockdown suppressed cell proliferation. We previously demonstrated a correlation between *ID1* expression levels and cell proliferation in different cell types (9). This inhibition was strongly associated with *ID1*-knockdown-induced c-myc down-regulation and p21 up-regulation. *ID1* can interact with these proteins directly or indirectly (24-26). Moreover, some reports indicated an association between *ID1* and AKT in breast cancer and oral squamous cell carcinoma (27, 28). Therefore, we next determined whether the effects of *ID1* in SGC cells involved the AKT pathway.

pAKT is known to be involved in cell proliferation in several tumor types (29, 30). We investigated whether pAKT (Thr308, Ser473) is involved in the *ID1*-associated proliferation of SGC cells. Inactivation of the AKT pathway induced by *ID1* knockdown coincided with changes in the expression of the cell cycle-related proteins p21, 27, as well as c-myc. This result was in agreement with previous reports

(31, 32). Moreover, a previous report indicated that AKT phosphorylation leads to ubiquitination and degradation of the oncoprotein c-myc (33). This result suggests that down-regulation of the AKT pathway could also be involved in the down-regulation of c-myc expression.

Recently, a study investigating the relationship between E-cadherin and *ID1* expression indicated that E-cadherin expression depends on *ID1* in breast cancer cells (34). This may explain the changes in cell morphology observed in cells with *ID1* knockdown in the present study. These morphological changes may have resulted from induction of the epithelial mesenchymal transition by *ID1* knockdown.

ID1 knockdown also reduced cell invasiveness. We speculate that *ID1* is at least indirectly associated with the regulation of invasion-associated genes. In prostate cancer cells, silencing of *ID1* induced expression of the cell cycle regulatory proteins p16 and p21 and triggered a change in MMP9 levels (35). We previously reported that, in human aggressive SGC cells (10) and in metastatic breast cancer cells (11), introduction of the progesterone receptor and treatment with progestin were sufficient to reduce *ID1* expression, down-regulate c-myc and MMP9 and up-regulate p21, thereby reducing the aggressive phenotype of the cells. The down-regulation of proteins, such as MMPs, could explain the effects of *ID1* knockdown on the reduction of SGC cell invasion. It was recently reported that inhibition of AKT activity induced cell motility in pancreatic adenocarcinoma cells (36). Other reports have indicated that the presence of IGF-binding proteins is associated with MMP9 tumor-promoting effects in breast cancer (37). Moreover, *ID1* has been reported to be an important mediator of metastasis (38).

In conclusion, the crosstalk among *ID1*, IGF-II and the AKT pathway was strongly associated with malignant phenotypes of SGCs. SGC is a highly lethal malignancy and development of new therapeutic interventions is urgently needed. We conclude that *ID1* knockdown decreases IGF-II expression in human SGCs and that SGC-secreted IGF-II mediates the effect of *ID1* on the AKT pathway.

Conflicts of Interest

The Authors declared no financial or other potential conflict of interest.

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References

- 1 Polyak K, Haviv I and Campbell IG: Co-evolution of tumor cells and their microenvironment. *Trends Genet* 25: 30-38, 2009.

- 2 Elkabets M, Gifford AM, Scheel C, Nilsson B, Reinhardt F, Bray MA, Carpenter AE, Jirstrom K, Magnusson K, Ebert BL, Pontén F, Weinberg RA and McAllister SS: Human tumors instigate granulosa-expressing hematopoietic cells that promote malignancy by activating stromal fibroblasts in mice. *J Clin Invest* 121: 784-799, 2011.
- 3 Zha J and Lackner MR: Targeting the insulin-like growth factor receptor-1R pathway for cancer therapy. *Clin Cancer Res* 16: 2512-2517, 2010.
- 4 Heidegger I, Pircher A, Klocker H and Massoner P: Targeting the insulin-like growth factor network in cancer therapy. *Cancer Biol Ther* 11: 701-707, 2011.
- 5 Imsumran A, Adachi Y, Yamamoto H, Li R, Wang Y, Min Y, Piao W, Nosho K, Arimura Y, Shinomura Y, Hosokawa M, Lee CT, Carbone DP and Imai K: Insulin-like growth factor-I receptor as a marker for prognosis and a therapeutic target in human esophageal squamous cell carcinoma. *Carcinogenesis* 28: 947-956, 2007.
- 6 Chava S, Mohan V, Shetty PJ, Manolla ML, Vaidya S, Khan IA, Waseem GL, Boddala P, Ahuja YR and Hasan Q: Immunohistochemical evaluation of p53, FHIT, and IGF-II gene expression in esophageal cancer. *Dis Esophagus* 25: 81-87, 2012.
- 7 Youssef A and Han VK: Low oxygen tension modulates the insulin-like growth factor-1 or -2 signaling via both insulin-like growth factor-1 receptor and insulin receptor to maintain stem cell identity in placental mesenchymal stem cells. *Endocrinology* 157: 1163-1174, 2016.
- 8 Fong S, Itahana Y, Sumida T, Singh J, Coppe JP, Liu Y, Richards PC, Bennington JL, Lee NM, Debs RJ and Desprez PY: Id-1 as a molecular target in therapy for breast cancer cell invasion and metastasis. *Proc Natl Acad Sci USA* 100: 13543-13548, 2003.
- 9 Sumida T, Murase R, Onishi-Ishikawa A, McAllister SD, Hamakawa H and Desprez PY: Targeting Id1 reduces proliferation and invasion in aggressive human salivary gland cancer cells. *BMC Cancer* 13: 141, 2013.
- 10 Sumida T, Itahana Y, Hamakawa H and Desprez PY: Reduction of human metastatic breast cancer cell aggressiveness on introduction of either form A or B of the progesterone receptor and then treatment with progestins. *Cancer Res* 64: 7886-7892, 2004.
- 11 Yoshimura T, Sumida T, Liu S, Onishi A, Shintani S, Desprez PY and Hamakawa H: Growth inhibition of human salivary gland tumor cells by introduction of progesterone (Pg) receptor and Pg treatment. *Endocr Relat Cancer* 14: 1107-1116, 2007.
- 12 Singh J, Murata K, Itahana Y and Desprez PY: Constitutive expression of the Id-1 promoter in human metastatic breast cancer cells is linked with the loss of NF-1/Rb/HDAC-1 transcription repressor complex. *Oncogene* 21: 1812-1822, 2002.
- 13 Higuchi K, Gen K, Izumida D, Kazeto Y, Hotta T, Takashi T, Aono H and Soyano K: Changes in gene expression and cellular localization of insulin-like growth factors 1 and 2 in the ovaries during ovary development of the yellowtail, *Seriola quinqueradiata*. *Gen Comp Endocrinol* 2016 Jan 4. pii S0016-6480(16): 30001-30006.
- 14 Kool MM, Galac S, van der Helm N, Corradini S, Kooistra HS and Mol JA: Insulin-like growth factor-phosphatidylinositol 3 kinase signaling in canine cortisol-secreting adrenocortical tumors. *J Vet Intern Med* 29: 214-224, 2015.
- 15 Li B, Tsao SW, Chan KW, Ludwig DL, Novosyadlyy R, Li YY, He QY and Cheung AL: Id1-induced IGF-II and its autocrine/endocrine promotion of esophageal cancer progression and chemoresistance--implications for IGF-II and IGF-IR-targeted therapy. *Clin Cancer Res* 20: 2651-2662, 2014.
- 16 Jia W, Feng YI, Sanders AJ, Davies EI and Jiang WG: Phosphoinositide-3-Kinase Enhancers, PIKES: Their Biological Functions and Roles in Cancer. *Anticancer Res* 36: 1103-1109, 2016.
- 17 Sato N, Kyakumoto S, Sawano K and Ota M: Proliferative signal transduction by epidermal growth factor (EGF) in the human salivary gland adenocarcinoma (HSG) cell line. *Biochem Mol Biol Int* 38: 597-606, 1996.
- 18 Myoken Y, Myoken Y, Okamoto T, Kan M, McKeehan WL, Sato JD and Takada K: Expression of fibroblast growth factor-1 (FGF-1), FGF-2 and FGF receptor-1 in a human salivary-gland adenocarcinoma cell line: evidence of growth. *Int J Cancer* 65: 650-657, 1996.
- 19 Sun XH, Copeland NG, Jenkins NA and Baltimore D: Id proteins Id1 and Id2 selectively inhibit DNA binding by one class of helix-loop-helix proteins. *Mol Cell Biol* 11: 5603-5611, 1991.
- 20 Morgenstern JP and Land H: Advanced mammalian gene transfer: high titre retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line. *Nucleic Acids Res* 18: 3587-3596, 1990.
- 21 Fisher SJ and Werb Z: The catabolism of extracellular matrix components. In: *Extracellular Matrix: A Practical Approach* (Haralson MA, Hassell JR (eds.). Oxford, United Kingdom, IRL Press Ltd., pp. 261-287, 1995.
- 22 LeRoith D and Roberts CT Jr.: The insulin-like growth factor system and cancer. *Cancer Lett* 195: 127-137, 2003.
- 23 Mukherjee A, Wilson EM and Rotwein P: Insulin-like growth factor (IGF) binding protein-5 blocks skeletal muscle differentiation by inhibiting IGF actions. *Mol Endocrinol* 22: 206-215, 2008.
- 24 Patel D and Chaudhary J: Increased expression of bHLH transcription factor E2A (TCF3) in prostate cancer promotes proliferation and confers resistance to doxorubicin induced apoptosis. *Biochem Biophys Res Commun* 422: 146-151, 2012.
- 25 Shin DH, Park JH, Lee JY, Won HY, Jang KS, Min KW, Jang SH, Woo JK, Oh SH and Kong G: Overexpression of Id1 in transgenic mice promotes mammary basal stem cell activity and breast tumorigenesis. *Oncotarget* 6: 17276-17290, 2015.
- 26 Geng H, Rademacher BL, Pittsnerbarger J, Huang CY, Harvey CT, Lafortune MC, Myrthue A, Garzotto M, Nelson PS, Beer TM and Qian DZ: ID1 enhances docetaxel cytotoxicity in prostate cancer cells through inhibition of p21. *Cancer Res* 70: 3239-3248, 2010.
- 27 Zhao J, Wang S, Liu N and Tang X: Correlation between the expression of Id-1 and hyperthermia-associated molecules in oral squamous cell carcinoma. *J Clin Pathol* 66: 758-763, 2013.
- 28 Lee JY, Kang MB, Jang SH, Qian T, Kim HJ, Kim CH, Kim Y and Kong G: Id-1 activates Akt-mediated Wnt signaling and p27(Kip1) phosphorylation through PTEN inhibition. *Oncogene* 28: 824-831, 2009.
- 29 Zhang Z, Zhang G, Kong C, Zhan B, Dong X and Man X: METTL13 is downregulated in bladder carcinoma and suppresses cell proliferation, migration and invasion. *Sci Rep* 6: 19261, 2016.

- 30 Peng H, Du B, Jiang H and Gao J: Over-expression of CHAF1A promotes cell proliferation and apoptosis resistance in glioblastoma cells *via* AKT/FOXO3a/Bim pathway. *Biochem Biophys Res Commun* 469: 1111-1116, 2016.
- 31 Guo B, Xie P, Su J, Zhang T, Li X and Liang G: Fangchinoline suppresses the growth and invasion of human glioblastoma cells by inhibiting the kinase activity of Akt and Akt-mediated signaling cascades. *Tumour Biol* 37: 2709-2719, 2016.
- 32 Zhang S, Lu Z, Mao W, Ahmed AA, Yang H, Zhou J, Jennings N, Rodriguez-Aguayo C, Lopez-Berestein G, Miranda R, Qiao W, Baladandayuthapani V, Li Z, Sood AK, Liu J, Le XF and Bast RC Jr: CDK5 regulates paclitaxel sensitivity in ovarian cancer cells by modulating AKT activation, p21Cip1- and p27Kip1-mediated G1 cell cycle arrest and apoptosis. *PLoS One* 10: e0131833, 2015.
- 33 Quan Y, Wang N, Chen Q, Xu J, Cheng W, Di M, Xia W and Gao WQ: SIRT3 inhibits prostate cancer by destabilizing oncoprotein c-MYC through regulation of the PI3K/Akt pathway. *Oncotarget* 6: 26494-26507, 2015.
- 34 Zhou Y, Ming J, Xu Y, Zhang Y and Jiang J: ER β 1 inhibits the migration and invasion of breast cancer cells through upregulation of E-cadherin in a Id1-dependent manner. *Biochem Biophys Res Commun* 457: 141-147, 2015.
- 35 Asirvatham AJ, Carey JP and Chaudhary J: ID1-, ID2-, and ID3-regulated gene expression in E2A positive or negative prostate cancer cells. *Prostate* 67: 1411-1420, 2007.
- 36 Smith AJ, Wen YA, Stevens PD, Liu J, Wang C and Gao T: PHLPP negatively regulates cell motility through inhibition of Akt activity and integrin expression in pancreatic cancer cells. *Oncotarget* 7: 7801-7815, 2016.
- 37 Park JH, Rasch MG, Qiu J, Lund IK and Egeblad M: Presence of insulin-like growth factor binding proteins correlates with tumor-promoting effects of matrix metalloproteinase 9 in breast cancer. *Neoplasia* 17: 421-433, 2015.
- 38 Weidle UH, Birzele F, Kollmorgen G and R ger R: Molecular Basis of Lung Tropism of Metastasis. *Cancer Genomics Proteomics* 132: 129-139, 2016.

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