Chem Biol Drug Des 2014; **84:** 578–584

Research Article

Down-Regulation of Asparagine Synthetase Induces Cell Cycle Arrest and Inhibits Cell Proliferation of Breast Cancer

Hongjian Yang[1,†,*], Xiangming He[1,†], Yabing Zheng[2], Weiliang Feng[1], Xianghou Xia[1], Xingfei Yu[1,*] and Zhiqiang Lin[3]

[1]Department of Breast Surgery, Zhejiang Cancer Hospital, 38 Guangji Road, Hangzhou 310022, China
[2]Department of Internal Oncology, Zhejiang Cancer Hospital, 38 Guangji Road, Hangzhou 310022, China
[3]Zhejiang Cancer Research Institute, Zhejiang Cancer Hospital, 38 Guangji Road, Hangzhou 310022, China
**Corresponding author: Hongjian Yang, hongjian_yang@163.com*; Xingfei Yu, *xingfeiyu@yeah.net*
[†]These authors contributed equally to this work.

Asparagine synthetase (ASNS) is deemed to be a promising therapeutic target for the treatment of several cancers, but its functional role in human breast cancer is still unknown. In this study, we employed RNA interference as an efficient tool to silence endogenous ASNS expression in breast cancer cell lines. The relationship between ASNS expression and breast cancer cell growth was investigated, and the therapeutic value of ASNS in breast cancer was further evaluated. Depletion of ASNS remarkably inhibited the proliferation and colony formation capacity of breast cancer cells and arrested cell cycle in the S phase. Our findings suggest that ASNS may contribute to breast cancer tumorigenesis and could be a potential therapeutic target in human breast cancer.

Key words: asparagine synthetase, breast cancer, cell proliferation, RNA interference

Received 29 December 2013, revised 14 March 2014 and accepted for publication 15 April 2014

Breast cancer ranks first as a cause of cancer death in women (1), and the incidence of breast cancer is estimated to increase by approximately 0.5% per year (2). During 2013 in the US, an estimated 232 340 new cases of invasive breast cancer were expected to be diagnosed in women (3). Despite enormous improvements in treatment for breast cancer, still one-fifth of patients eventually die of this disease (1). As a result, these facts support the urgent need for the identification of biomarkers as early diagnostic and therapeutic targets in human breast cancer.

Asparagine synthetase (ASNS) is encoded by the *asparagine synthetase (ASNS)* gene and catalyzes the biosynthesis of L-asparagine (4). Previous studies demonstrated that ASNS expression is related to oncogenesis triggered by mutated p53 and resistance of leukemia cells to L-asparaginase (5–12), which has been commonly used as active ingredient to treat acute lymphoblastic leukemia (ALL) and some forms of acute myeloblastic leukemia (AML) (13). Cui *et al.* (14) reported that overexpression of ASNS in pancreatic cancer cells could induce resistance to apoptosis triggered by cisplatin and carboplatin, whereas ASNS contributes to cisplatin sensitivity by potentiating cisplatin-induced DNA damage and apoptosis in nasopharyngeal carcinoma cells (15). ASNS plays an important role in the cell cycle arrest of cancer cells and coordinates with L-asparaginase activity in leukemia and ovarian cancer (16–18). Sircar *et al.* (19) showed that ASNS is up-regulated in castration-resistant prostate cancer (CRPC), and the disruption of asparagine by ASNS inhibitors could be a novel therapeutic approach for CRPC. Recently, it is reported that the expression of ASNS is an independent factor affecting the survival of hepatocellular carcinoma (HCC) patients, and low ASNS expression in HCC was correlated with worse surgical outcomes (20). Moreover, high expression of ASNS has also been associated with biological aggressiveness of gliomas (21). However, the relationship between endogenous ASNS expression and breast cancer development remains largely unknown.

In this study, we employed a RNA interference lentivirus system to knock down ASNS expression in human breast cancer cell lines ZR-75-30 and MDA-MB-231. Subsequently, cell proliferation, colony formation, and cell cycle assays were conducted to explore the potential function of ASNS in breast cancer cells. To our knowledge, this is the first presentation providing evidence that down-regulation of ASNS is sufficient to inhibit the growth of breast cancer cells, which may ensure its important role as a promising therapeutic target in breast cancer.

Materials and Methods

Cell culture

Human breast cancer cell lines ZR-75-30, MDA-MB-231 and human embryonic kidney cell line HEK293T were

 doi: 10.1111/cbdd.12348

obtained from Shanghai Institute of Cell Biology, the Chinese Academy of Sciences. ZR-75-30 cells were cultured in RPMI-1640 (Hyclone, Logan, UT, USA) supplemented with 10% FBS (Hyclone) at 37 °C in a humidified atmosphere of 5% CO_2. MDA-MB-231 and HEK293T cells were maintained in DMEM (Hyclone) supplemented with 10% FBS at 37 °C in a humidified atmosphere of 5% CO_2.

Construction of recombinant lentivirus

The following oligonucleotides were synthesized. The negative control small interfering RNA (siRNA) was 5′-TTCTCCGAACGTGTCACGT-3′. An shRNA sequence (5′-GTGAACATTATGAAGTCCTTTCTCGAGAAAGGACTTCATA ATGTTCACTTTTT-3′) for human ASNS gene (NM_001673) was screened and validated to be a candidate shRNA. The stem-loop-stem oligos (shRNAs) were synthesized, annealed and ligated into the AgeI/EcoRI-linearized pFH-L vector (Shanghai Hollybio, China). The shRNA-expressing lentiviral vectors were confirmed by DNA sequencing. The generated plasmids were named as pFH-L-shASNS and pFH-L-shCon.

HEK293T cells (1.0×10^6 cells/dish) were seeded into 10-cm dishes and cultured for 24 h to reach 70–80% confluence. Two hours before transfection, the medium was replaced with serum-free DMEM. Plasmids including 10 μg of pFH-L-shASNS or pFH-L-shCon, 7.5 μg of packaging vector pHelper 1.0 and 5 μg of expression plasmid pHelper 2.0 were added to 0.95 mL of Opti-MEM and 50 μL of Lipofectamine 2000. The mixture was added to the cells and incubated for 8 h before replacing the medium with 10 mL of DMEM medium (with 10% PBS). The supernatant was collected 48 h after transfection, and the lentiviral particles were harvested by ultracentrifugation (4000 × *g*) at 4 °C for 10 min. The collected virus particles were filtered through a 45-μm filter, and the filtrate was centrifuged (4000 × *g* at 4 °C for 15 min) to collect the viral concentrate. ZR-75-30 or MDA-MB-231 cells were cultured in 6-well plates and treated with recombinant lentiviruses (Lv-shASNS or Lv-shCon) at an MOI of 35 or 20, respectively. The infection efficiency was determined through counting green fluorescence protein (GFP)-expressing cells under fluorescence microscope 96 h after infection.

RNA extraction and real-time PCR

ZR-75-30 and MDA-MB-231 cells were precultured and infected with recombinant lentivirus for 5 days, respectively. Total RNA was extracted using Trizol reagent (Gibco RL, Grand Island, NY, USA) according to the manufacturer's instruction. cDNA was synthesized using SuperScript II Reverse Transcriptase 200 U/mL (Invitrogen, Carlsbad, CA, USA). ASNS mRNA expression was evaluated by real-time PCR on the Bio-Rad Connect Real-Time PCR platform (Bio-Rad, Hercules, CA, USA) with SYBR Green PCR core reagents. β-actin was applied as an internal reference. The primers used were as follows, ASNS: 5′-TGCTTACGCCCAGATTTTCT-3′ (forward) and 5′-AAAA CGGAATGCATCTGGAC-3′ (reverse); β-actin: 5′-GTGGAC ATCCGCAAAGAC-3′ (forward) and 5′-AAAGGGTGTAAC GCAACTA-3′ (reverse). The reaction procedure was initial denaturation at 95 °C for 1 min and 40 cycles of denaturation at 95 °C for 5 seconds followed by annealing extension at 60 °C for 20 seconds. Results are presented as CT values, defined as the threshold PCR cycle number at which an amplified product is first detected. The average CT was calculated for both ASNS and β-actin, and ΔCT was determined as the mean of the triplicate CT values for ASNS minus the mean of the triplicate CT values for β-actin.

Western blot analysis

Lentivirus-transduced cells were washed twice with ice-cold PBS and lysed in 2 × SDS sample buffer (100 mM Tris–HCl (pH 6.8), 10 mM EDTA, 4% SDS, 10% Glycine). Equal amount of proteins (30 μg) were loaded and separated on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). Blots were blocked and then probed with primary antibodies, rabbit anti-ASNS (1:3000 dilution; Proteintech Group Inc., Chicago, IL, USA) or rabbit anti-GAPDH (1:3000 dilution; Proteintech Group Inc.) overnight at 4 °C. After washing, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies and visualized by super ECL detection reagent (Applygen, Beijing, China).

MTT viability assay

After 96 h of infection, ZR-75-30 (2×10^3 cells/well) and MDA-MB-231 (2.5×10^3 cells/well) were recultured in 96-well plates, respectively. Cell viability was analyzed using the MTT assay. The number of viable cells was measured at daily intervals (days 1, 2, 3, 4 and 5). At each time point, 20 μL of the MTT solution (5 mg/mL) was added to each well, and then the samples were incubated for 4 h. The formed formazan crystals were dissolved in 100 μL of acidic isopropanol (10% SDS, 5% isopropanol and 0.01 M HCl), and the optical density was measured using an ELISA plate reader at 595 nm.

Colony formation assay

After 96 h of incubation, cells were recultured in 6-well plates with a concentration of 500 cells per well and allowed to form natural colonies. After 5 or 9 days of culture, ZR-75-30 or MDA-MB-231 cells were washed with PBS and fixed with 4% paraformaldehyde for 30 min at room temperature. Subsequently, the fixed cells were stained with crystals purple for 10 min, washed with ddH_2O and air-dried. The total number of colonies (>50 cells/colony) was counted using fluorescence microscopy.

Flow cytometry analysis

After 72 h of infection, MDA-MB-231 cells were inoculated into 6-cm dishes at a density of 2×10^5 cells per dish. After 48 h of culture at 37 °C, cells were collected and fixed with 70% cold ethanol for 30 min at 4 °C. Cells were then harvested by centrifugation, resuspended in PBS containing 100 μg/mL of DNase-free RNase and 40 μg/mL propidium iodide (PI) and incubated for 1 h at room temperature. The cell suspension was next filtered through a 50-μm nylon mesh, and 1×10^4 stained cells were analyzed by FAC Scan flow cytometer (Becton Dickinson, San Jose, CA, USA) in accordance with the manufacturer's guidelines.

Statistical analysis

All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). The differences between groups were evaluated using Student's t-test, and all data were expressed as mean ± SD of three independent experiments. Statistical significant difference was accepted at $p < 0.05$.

Results

Expression levels of ASNS could be specifically down-regulated by siRNA

ZR-75-30 and MDA-MB-231 cell lines were used to investigate loss of function in this study. Both cell lines were cultured and infected with Lv-shCon or Lv-shASNS. Non-infected cells were deemed as negative control (Con). GFP tag was embedded in lentivirus to provide visualized confirmation of transfection efficiency. It was confirmed that over 90% of cells were successfully infected with respective lentivirus, and the transfection rate was satisfying (Figure 1). To verify that the ASNS gene was silenced by the Lv-shASNS vector, the expression levels of endogenous ASNS mRNA and protein in both cell lines were analyzed using real-time PCR (Figure 2A and C) and western blot (Figure 2B and D), respectively. The expression levels of ASNS were remarkably down-regulated ($p < 0.01$) in the absence of ASNS compared with those in Lv-shCon-infected cells. The knockdown efficiency was 90.9% and 66.9% in the ZR-75-30 and MDA-MB-231 cells, respectively. Therefore, our constructed lentivirus could be an efficient tool to reduce ASNS expression in breast cancer cells.

Deprivation of ASNS remarkably inhibited the growth of breast cancer cells

To better understand the role of ASNS in breast cancer development, we examined the alteration of cell proliferation after lentivirus infection. MTT viability assay yields wonder testing sensitivity and good dynamic range. Cell proliferation rate was determined utilizing MTT assay after 5-day incubation. Line charts in Figure 3 indicated that Lv-shCon-infected cells had no obvious difference to control cells, but a significant proliferation inhibition was observed in ASNS-silenced cells ($p < 0.001$).

Meanwhile, the colony formation assay in both ZR-75-30 and MDA-MB-231 cells demonstrated the down-regulation of ASNS could result in a significant reduction in colony formation capacity (Figure 4). The colony formed was remarkably smaller, and the colonies numbers were statistically fewer in Lv-shASNS groups compared with Lv-shCon groups ($p < 0.001$). Whereas there was no noticeable difference between Lv-shCon groups and Con groups. Collectively, knockdown of ASNS significantly inhibited the proliferation and colony formation capacity of breast cancer cells.

S-phase cell cycle arrest was induced by down-regulation of ASNS

To elucidate the mechanism underlying the inhibition of cell growth, we examined the cell cycle distribution of MDA-MB-231 cells with three different treatments (Con,

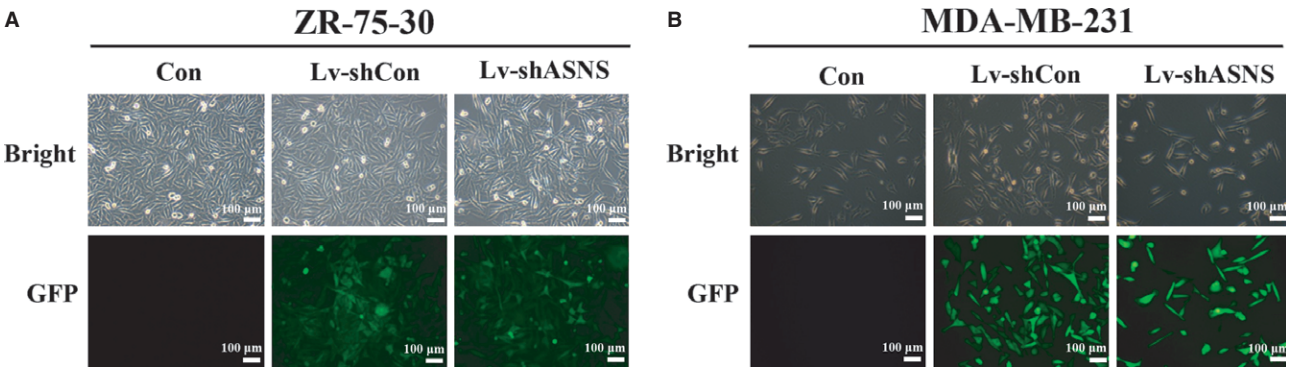

Figure 1: Determination of infection efficiency in breast cancer cells. Representative images of ZR-75-30 (A) and MDA-MB-231 (B) cells infected with lentivirus at MOI of 35 or 20, respectively (scale bar: 100 μm). Con: non-infected, Lv-shCon: non-silencing lentivirus, Lv-shASNS: ASNS-silencing lentivirus.

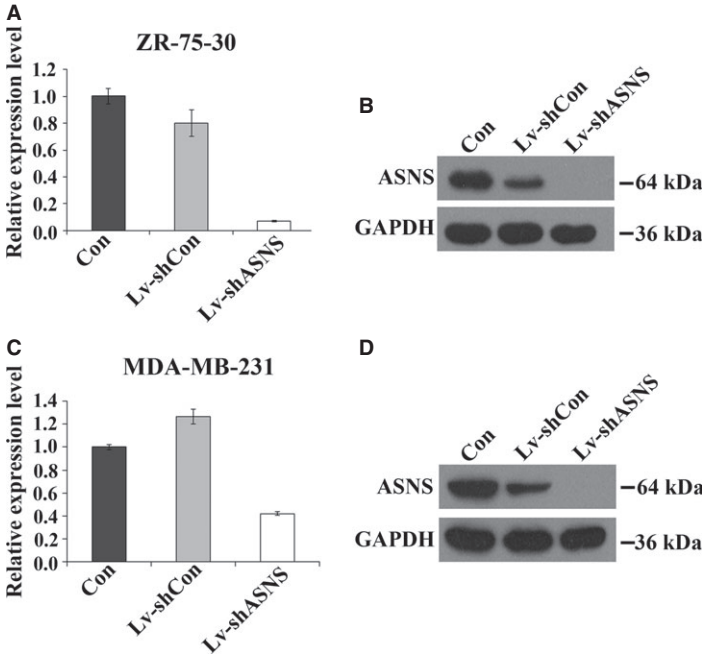

Figure 2: Expression of ASNS was knocked down of by RNAi. qRT-PCR analysis of ASNS mRNA in ZR-75-30 (A) and MDA-MB-231 (C) cells with three different treatment (Con, Lv-shCon, Lv-shASNS). β-actin was used as an internal gene. Western blot analysis of ASNS protein in ZR-75-30 (B) and MDA-MB-231 (D) cells with three different treatment (Con, Lv-shCon, Lv-shASNS). GAPDH was used as an internal control. **p < 0.01, compared to Lv-shCon.

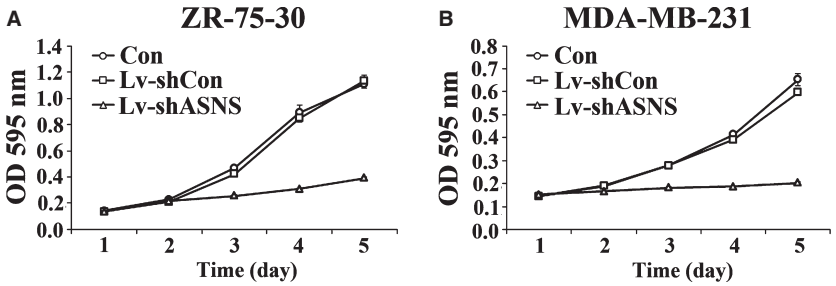

Figure 3: Depletion of ASNS suppressed the proliferation of breast cancer cells. The monolayer proliferation rate of ZR-75-30 (A) and MDA-MB-231 (B) cells from triplicate groups were determined by MTT assay. ***p < 0.001, compared to Lv-shCon.

Lv-shCon, Lv-shASNS). As shown in Figure 5, compared with Lv-shCon-infected cells, the number of cells in the S phase was significantly increased ($p < 0.001$), whereas the percentages of cells in the G0/G1 phase and G2/M phase were concomitantly decreased in MDA-MB-231 cells infected with Lv-shASNS. These results indicated that depletion of ASNS could induce cell cycle arrest in the S phase, which might contribute to the disruption of cell growth.

Discussion

In this study, we employed RNA interference to examine the correlation of ASNS expression with cell growth in human breast cancer. Our data showed that the deprivation of endogenous ASNS expression led to inhibited cell proliferation, impaired colony formation and blocked cell cycle progression. These findings advance our understanding of the basic biological mechanism of ASNS in human breast cancer cells *in vitro* and further reveal the critical role of ASNS in managing cell growth and cell cycle progression.

It is known that ASNS knockdown may lead to the accumulation of aspartate and glutamine, which are essential components in the biosynthesis of purines and pyrimidines. Amino acid availability modulates serial fundamental progresses of gene expression, transcription factor recruitment, mRNA processing, and translation (22). Previous studies have demonstrated that ASNS is necessary for G1 progression in hamster BHK ts11 cells, and the loss of ASNS activity can lead to cell cycle arrest (23,24). Our studies corroborate these previously reported demonstrations: down-regulation of ASNS could severely suppress the urinary cancer cell proliferation and colony formation. Moreover, Zhang *et al.* (20) showed the overexpression of ASNS could inhibit the migration capacity of cells in

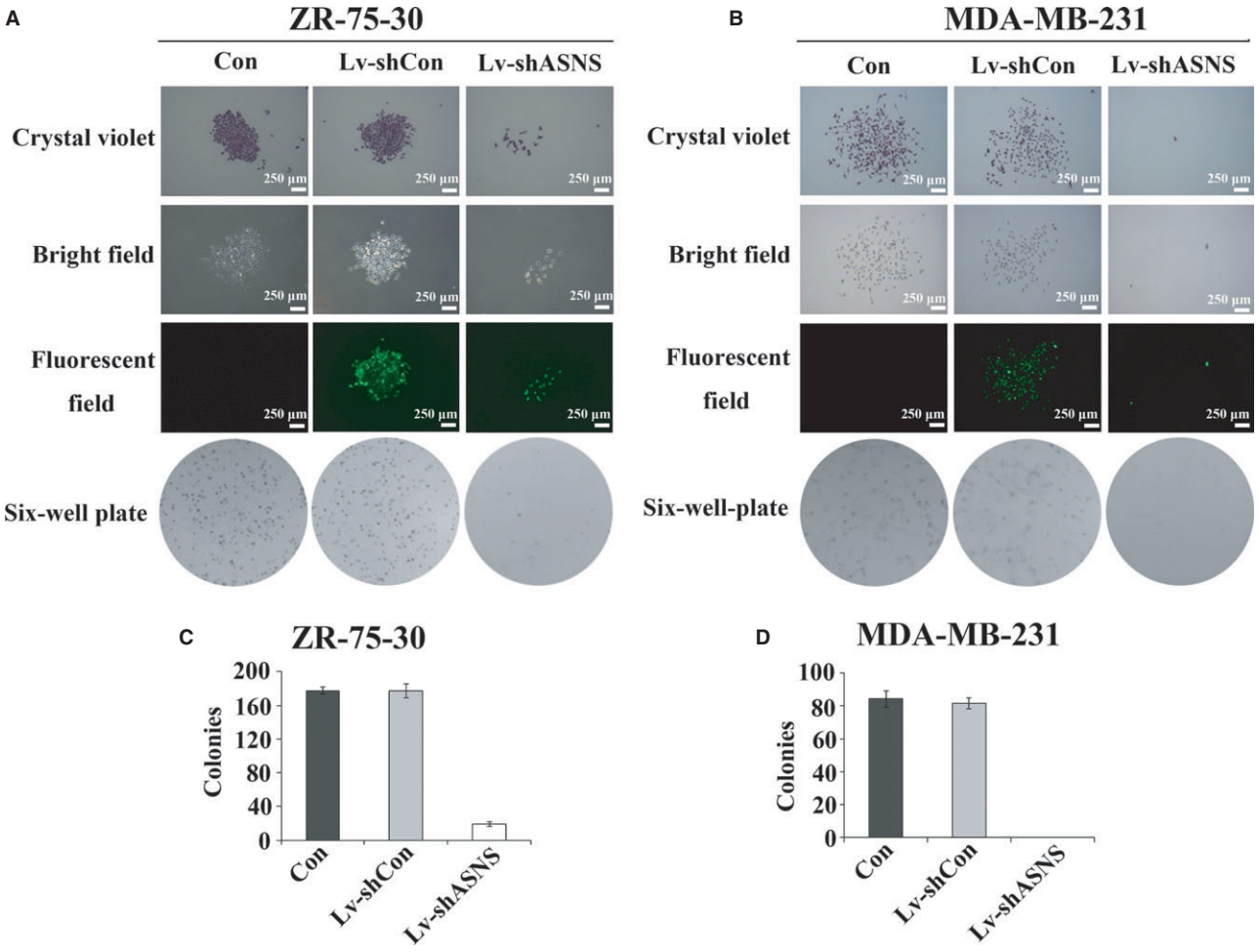

Figure 4: Knockdown of ASNS inhibited the colony formation of breast cancer cells. Representative images of colonies formed in ZR-75-30 (A) and MDA-MB-231 (B) cells with three different treatment (Con, Lv-shCon, Lv-shASNS) (scale bar: 250 μm). Upper three rows represent images of single colony. The lowest row represents full vision of 6-well plates under microscope. Statistical analysis of colonies numbers in ZR-75-30 (C) and MDA-MB-231 (D) cells utilizing crystal violet staining. ***$p < 0.001$, compared to Lv-shCon.

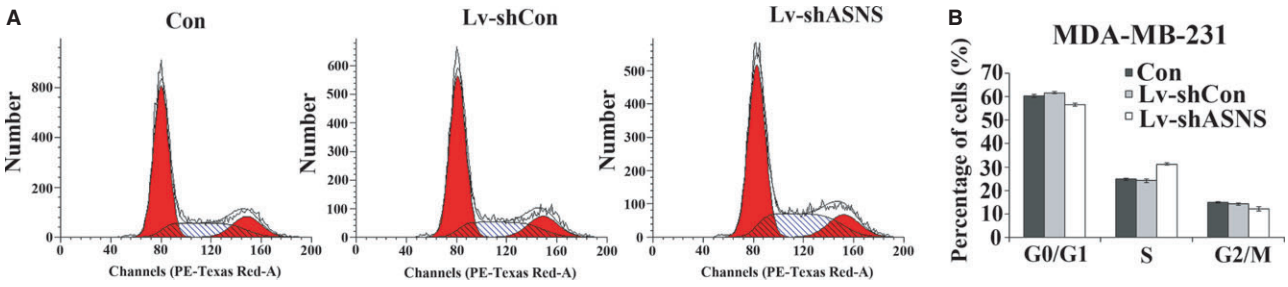

Figure 5: Down-regulation of ASNS induced cell cycle arrest. (A) Cell cycle distribution of MDA-MB-231 cells was analyzed by flow cytometry. (B) The population of cells in S phase was remarkably increased, accompanied by reduction in G0/G1 phase and G2/M phase. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to Lv-shCon.

human hepatocellular cancer (HCC). Further investigation should be considered when extending the usage of ASNS.

Similarly, one of the amino acid catalyzing enzyme, L-asparaginase, has been employed in the treatment of acute lymphoblastic leukemia (ALL) for almost 50 years (25,26). It is an enzyme chemotherapy drug that interferes with growing cancer cells, especially leukemia cells and normal cells are less affected (13). Li *et al.* (27) reported that the expression levels of ASNS were significantly increased in

human leukemic cell lines when cocultured with L-asparaginase. And it is generally accepted that down-regulated ASNS expression improves the therapeutic effects of L-asparaginase on pancreatic and ovarian tumors (16,17,28) and further extended on HCC patients (20). In this study, in order to determine whether ASNS could be a promising therapeutic target in breast cancer, we employed lentivirus-mediated shRNA to silence ASNS expression and showed that ASNS knockdown could remarkably inhibit breast cancer cell growth along with S phase arrest, which are consistent with reported demonstrations. Additional testing of ASNS down-regulation in combination with L-asparaginase on breast cancer cells to improve tumor killing is worthy of further consideration, particularly when the combination of ASNS silencing and L-asparaginase was not acutely toxic in whole animal.

Insulin-like growth factor (IGF) is a key regulator in tumorigenesis of breast cancer. It was approved that overexpression of IGF can potently promote cellular proliferation rate and the efficiency of tumor formation in mouse (29). ASNS, which plays an essential role in amino acid synthesis and import, can be up-regulated by the overexpression of IGF (29). ASNS is the key enzyme in the biosynthesis pathway of asparagine, which is an essential precursor for the synthesis of pyrimidine bases (30), so the down-regulated ASNS expression could reduce the intracellular pyrimidine bases pool in breast cancer cells, and as a sequence, the proliferation of cancer cells has been prohibited.

Collectively, the majority of genes constitutively regulated by ASNS are critically involved in amino acids transport and metabolism, synthesis of nucleic acid bases, and protein biosynthesis. All of these mentioned processes are typical basic requirements for accelerated proliferation and growth in tumor cells. The results suggest that ASNS modulates breast cancer cell proliferation by regulating cell cycle progression, which ensures us a useful starting point in the discovery of promising biomarkers for human breast cancer.

Acknowledgments

The authors are thankful for the financial support from the Natural Science Foundation of Zhejiang Province, China (No. LY12H16030), Foundation of Science Technology Department of Zhejiang Province, China (No. 2011C23043), Foundation of Health Department of Zhejiang Province, China (No. 2011BCA015), Traditional Chinese Medical Research Foundation of Zhejiang Province, China (No. 2011ZA016).

Conflict of Interest

None.

References

1. Jemal A., Bray F., Center M.M., Ferlay J., Ward E., Forman D. (2011) Global cancer statistics. CA Cancer J Clin;61:69–90.
2. Jemal A., Siegel R., Ward E., Murray T., Xu J., Smigal C., Thun M.J. (2006) Cancer statistics, 2006. CA Cancer J Clin;56:106–130.
3. American Cancer Society. Cancer Facts & Figures. Atlanta, GA: The Society; p. v.
4. Richards N.G., Schuster S.M. (1998) Mechanistic issues in asparagine synthetase catalysis. Adv Enzymol Relat Areas Mol Biol;72:145–198.
5. Prager M.D., Bachynsky N. (1968) Asparagine synthetase in asparaginase resistant and susceptible mouse lymphomas. Biochem Biophys Res Commun;31:43–47.
6. Hutson R.G., Kitoh T., Moraga Amador D.A., Cosic S., Schuster S.M., Kilberg M.S. (1997) Amino acid control of asparagine synthetase: relation to asparaginase resistance in human leukemia cells. Am J Physiol;272: C1691–C1699.
7. Amylon M.D., Shuster J., Pullen J., Berard C., Link M.P., Wharam M., Katz J., Yu A., Laver J., Ravindranath Y., Kurtzberg J., Desai S., Camitta B., Murphy S.B. (1999) Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. Leukemia;13:335–342.
8. Aslanian A.M., Fletcher B.S., Kilberg M.S. (2001) Asparagine synthetase expression alone is sufficient to induce l-asparaginase resistance in MOLT-4 human leukaemia cells. Biochem J;357:321–328.
9. Pui C.H., Relling M.V., Campana D., Evans W.E. (2002) Childhood acute lymphoblastic leukemia. Rev Clin Exp Hematol;6:161–180; discussion 200-2.
10. Zwaan C.M., Kaspers G.J., Pieters R., Hahlen K., Janka-Schaub G.E., van Zantwijk C.H., Huismans D.R., de Vries E., Rots M.G., Peters G.J., Jansen G., Creutzig U., Veerman A.J. (2002) Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. Blood;99:245–251.
11. Richards N.G., Kilberg M.S. (2006) Asparagine synthetase chemotherapy. Annu Rev Biochem;75:629–654.
12. Scian M.J., Stagliano K.E., Deb D., Ellis M.A., Carchman E.H., Das A., Valerie K., Deb S.P., Deb S. (2004) Tumor-derived p53 mutants induce oncogenesis by transactivating growth-promoting genes. Oncogene;23:4430–4443.
13. Apostolidou E., Swords R., Alvarado Y., Giles F.J. (2007) Treatment of acute lymphoblastic leukaemia: a new era. Drugs;67:2153–2171.
14. Cui H., Darmanin S., Natsuisaka M., Kondo T., Asaka M., Shindoh M., Higashino F., Hamuro J., Okada F.,

Kobayashi M., Nakagawa K., Koide H., Kobayashi M. (2007) Enhanced expression of asparagine synthetase under glucose-deprived conditions protects pancreatic cancer cells from apoptosis induced by glucose deprivation and cisplatin. Cancer Res;67:3345–3355.
15. Liu R.Y., Dong Z., Liu J., Zhou L., Huang W., Khoo S.K., Zhang Z., Petillo D., Teh B.T., Qian C.N., Zhang J.T. (2013) Overexpression of asparagine synthetase and matrix metalloproteinase 19 confers cisplatin sensitivity in nasopharyngeal carcinoma cells. Mol Cancer Ther;12:2157–2166.
16. Lorenzi P.L., Llamas J., Gunsior M., Ozbun L., Reinhold W.C., Varma S., Ji H., Kim H., Hutchinson A.A., Kohn E.C., Goldsmith P.K., Birrer M.J., Weinstein J.N. (2008) Asparagine synthetase is a predictive biomarker of L-asparaginase activity in ovarian cancer cell lines. Mol Cancer Ther;7:3123–3128.
17. Lorenzi P.L., Reinhold W.C., Rudelius M., Gunsior M., Shankavaram U., Bussey K.J., Scherf U. *et al.* (2006) Asparagine synthetase as a causal, predictive biomarker for L-asparaginase activity in ovarian cancer cells. Mol Cancer Ther;5:2613–2623.
18. Fiocca R., Necchi V., Sommi P., Ricci V., Telford J., Cover T.L., Solcia E. (1999) Release of Helicobacter pylori vacuolating cytotoxin by both a specific secretion pathway and budding of outer membrane vesicles. Uptake of released toxin and vesicles by gastric epithelium. J Pathol;188:220–226.
19. Sircar K., Huang H., Hu L., Cogdell D., Dhillon J., Tzelepi V., Efstathiou E., Koumakpayi I.H., Saad F., Luo D., Bismar T.A., Aparicio A., Troncoso P., Navone N., Zhang W. (2012) Integrative molecular profiling reveals asparagine synthetase is a target in castration-resistant prostate cancer. Am J Pathol;180:895–903.
20. Zhang B., Dong L.W., Tan Y.X., Zhang J., Pan Y.F., Yang C., Li M.H., Ding Z.W., Liu L.J., Jiang T.Y., Yang J.H., Wang H.Y. (2013) Asparagine synthetase is an independent predictor of surgical survival and a potential therapeutic target in hepatocellular carcinoma. Br J Cancer;109:14–23.
21. Panosyan E.H., Wang Y., Xia P., Lee W.N., Pak Y., Laks D.R., Lin H.J., Moore T.B., Cloughesy T.F., Kornblum H.I., Lasky J.L. 3rd. (2014) Asparagine depletion potentiates the cytotoxic effect of chemotherapy against brain tumors. Mol Cancer Res. [Epub ahead of print].
22. Kilberg M.S., Pan Y.X., Chen H., Leung-Pineda V. (2005) Nutritional control of gene expression: how mammalian cells respond to amino acid limitation. Annu Rev Nutr;25:59–85.
23. Greco A.I.M., Basilico C. (1987) Molecular cloning of a gene that is necessary for G1 progression in mammalian cells. Proc Natl Acad Sci USA;84:1565–1569.
24. Gong S.S., Basilico C. (1990) A mammalian temperature-sensitive mutation affecting G1 progression results from a single amino acid substitution in asparagine synthetase. Nucleic Acids Res;18:3509–3513.
25. Haskell C.M. (1981) L-Asparaginase: human toxicology and single agent activity in nonleukemic neoplasms. Cancer Treat Rep;65(Suppl 4):57–59.
26. Verma N., Kumar K., Kaur G., Anand S. (2007) L-asparaginase: a promising chemotherapeutic agent. Crit Rev Biotechnol;27:45–62.
27. Li B.S., He Y.Y., Luo C.Y., Jiang H., Shen S.H., Jiang L.M., Zhang B., Gu L.J. (2010) Relationship between asparagine synthetase expression level and cell sensitivity to L-asparaginase in human leukemic cell lines. Zhongguo Shi Yan Xue Ye Xue Za Zhi;18:559–563.
28. Dufour E., Gay F., Aguera K., Scoazec J.Y., Horand F., Lorenzi P.L., Godfrin Y. (2012) Pancreatic tumor sensitivity to plasma L-asparagine starvation. Pancreas;41:940–948.
29. Pacher M., Seewald M.J., Mikula M., Oehler S., Mogg M., Vinatzer U., Eger A., Schweifer N., Varecka R., Sommergruber W., Mikulits W., Schreiber M. (2007) Impact of constitutive IGF1/IGF2 stimulation on the transcriptional program of human breast cancer cells. Carcinogenesis;28:49–59.
30. Berg J.M., Tymoczko J.L., Stryer L., Stryer L. (2002) Biochemistry, 5th edn. New York, NY: W.H. Freeman.