European Review for Medical and Pharmacological Sciences 2020; 24: 5946-5952

LncRNA CDKN2BAS aggravates the progression of ovarian cancer by positively interacting with GAS6

H.-M. WANG, S.-L. SHEN, N.-M. LI, H.-F. SU, W.-Y. LI

Department of Obstetrics and Gynecology, Jinan City People's Hospital, Jinan, China

Abstract. – OBJECTIVE: The aim of this study was to elucidate the role of long non-coding RNA (lncRNA) CDKN2BAS in aggravating the progression of ovarian cancer via binding growth arrest-specific 6 (GAS6).

PATIENTS AND METHODS: The relative levels of CDKN2BAS and GAS6 in ovarian cancer and normal ovarian tissues were detected. In addition, their levels in ovarian cancer cases with different FIGO stages and pathological grades were detected. Pearson correlation test was applied for assessing the correlation between CDKN2BAS and GAS6 levels in ovarian cancer tissues. The roles of CDKN2BAS and GAS6 in mediating proliferative and migratory potentials in HEY and SKOV-3 cells were examined by Cell Counting Kit-8 (CCK-8) and transwell assay, respectively. Subcellular distribution of CDKN2BAS was explored. CDKN2BAS-GAS6 interaction was evaluated by RIP (RNA immunoprecipitation) assay.

RESULTS: CDKN2BAS was upregulated in ovarian cancer tissues, especially those with advanced FIGO stage and high pathological grade. It displayed diagnostic potential in ovarian cancer. CDKN2BAS level was positively correlated to that of GAS6 in ovarian cancer tissues. It was mainly expressed in the cytoplasm and could be interacted with GAS6. The overexpression of CDKN2BAS enhanced proliferative and migratory potentials in HEY and SKOV-3 cells. The knockdown of GAS6 partially abolished the regulatory effects of CDKN2BAS on promoting proliferative and migratory potentials in ovarian cancer.

CONCLUSIONS: LncRNA CDKN2BAS is upregulated in ovarian cancer. By positively interacting with GAS6, CDKN2BAS triggers the progression of ovarian cancer.

Key Words:
LncRNA CDKN2BAS, GAS6, Ovarian cancer.

Introduction

Ovarian cancer is a prevalent female malignant tumor. The detective rate of advanced ovarian cancer is high because of the anatomic location of the ovaries, insidious onset and atypical symptoms in the early phase. In addition, rapid progression, low therapeutic efficacy and high recurrent rate result in the high mortality of ovarian cancer[1,2]. It is of significance to clarify the molecular mechanism of ovarian cancer and to develop effective biomarkers for diagnosis and treatment.

Long non-coding RNAs (LncRNAs) are non-coding RNAs containing more than 200 nucleotides[3]. They were used to be considered as transcript noises[4]. With the progressed research, it is found that lncRNAs are able to epigenetically, transcriptionally, and post-transcriptionally target genes[5]. LncRNAs are widely involved in tumor progression by influencing tumor cell phenotypes and angiogenesis[6].

LncRNA CDKN2BAS exerts a vital role in cell proliferation and apoptosis, and extracellular matrix remodeling[7,8]. Its expression is closely associated with susceptibilities to human diseases (i.e. coronary artery diseases and diabetes)[7]. Besides, CDKN2BAS has been identified to be a critical regulator in brain tumors, breast cancer, and myeloblastoma[8-10].

GAS6 (growth arrest-specific 6; 75 kDa) is a secreted protein that was initially discovered in the NIH 3T3 mouse embryo fibroblast cell line in 1988[11]. GAS6 exerts a negative role in cell growth. Later, GAS6 has been found to be widely expressed in organs and somatic cells[12]. It is reported that GAS6 is a vital regulator in the early phase of ovarian cancer progression[13]. LINC00565/GAS6 axis drives the progression of ovarian cancer[14]. This study aims to explore the role of CDKN2BAS/GAS6 axis in the progression of ovarian cancer. Our findings may provide novel ideas in the clinical treatment of ovarian cancer.

Patients and Methods

Sample Collection

Ovarian cancer tissues (n=44) and normal ovarian tissues (n=16) were collected from Jinan City

Corresponding Author: Wenying Li, BM; e-mail: lwy0707007@163.com

People's Hospital from May 2017 to July 2019. They were pathologically confirmed and stored at -80°C. All the patients involved in the study were initially treated with surgery and none of recruited subjects were treated with preoperative radiotherapy nor chemotherapy. The tissues of the epithelial ovarian cancer group and the normal ovarian group were confirmed by postoperative pathological diagnosis. This study was approved by the Ethics Committee of Jinan City People's Hospital. Signed written informed consents were obtained from all participants before the study.

Cell Culture and Transfection

Ovarian cancer cell lines (A2780, HO8910, HEY, and SKOV-3) and the ovarian epithelial cell line (IOSE-80) were provided by American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) in a 5% CO_2 incubator at 37°C. 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin were applied in the culture medium.

Transfection plasmids were provided by Genechem, Co., Ltd. (Shanghai, China). The cells were cultured to 60-70% density, and transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in serum-free medium. 6 hours later, the complete medium was replaced.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol (Invitrogen, Carlsbad, CA, USA) was applied for lysing cells or tissues and extracting total RNAs. Reverse transcription of RNAs was performed by the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) and complementary deoxyribose nucleic acid (cDNA) was sent for qRT-PCR. The relative level of the target was calculated using $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows. CDKN2BAS: F: 5′-TGCCGGAGCTGTCGACCC-3′, R: 5′-TTTGATCTCTGCTGTTGAATCAGAATG-3′; GAS6: F: 5′-CCGGAGCGAGGACTGTATCATCT-3′, R: 5′-ACTTCCCAGGTTGATTCAGTCCC-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): F: 5′-CTCCTGCATGCCACGGA-3′, R: 5′-AGACCCTTACAGTTAGTCGT-3′.

Cell Counting Kit-8 (CCK-8) Assay

Cells were inoculated into 96-well plates with 1×10^3 cells per well. At the appointed time points, 10 μL of CCK-8 solution (Dojindo Molecular Technolgies, Kumamoto, Japan) was added in each well. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Transwell Assay

100 μL of suspension (1×10^5 cells/ml) was inoculated in the upper insert of a transwell chamber (Millipore, Billerica, MA, USA), which was inserted in a 24-well plate with 500 μL of medium containing 10% FBS in the bottom. 48 hours later, bottom cells were reacted with 15-min methanol, 20-min crystal violet, and captured using a microscope. The number of migratory cells was counted in 10 random fields per sample (magnification 200×).

Subcellular Distribution Analysis

PARIS kit (Invitrogen, Carlsbad, CA, USA) was used for isolating cytoplasmic and nuclear fractions. RNAs in cellular components were detected by qRT-PCR. U6 and GAPDH were the internal references of nucleus and cytoplasm, respectively.

RIP (RNA Immunoprecipitation)

The cells were collected for incubating with input, anti-IgG or anti-CDKN2BAS at 4°C overnight. Intracellular proteins were captured, followed by obtaining the protein-RNA complex. After digestion in proteinase K, protein fraction was cleared. The remaining immunoprecipitant RNAs were subjected to qRT-PCR.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was used for data analyses. Data were expressed as mean ± standard deviation (SD). Receiver operating characteristic (ROC) curves were depicted for assessing the diagnostic potential of CDKN2BAS in ovarian cancer. The differences between the two groups were analyzed by the *t*-test. The relationship between the expression levels of the two genes was analyzed by Pearson correlation test. $p<0.05$ was considered as statistically significant.

Results

CDKN2BAS was Upregulated in Ovarian Cancer Tissues

Compared with controls, CDKN2BAS was upregulated in ovarian cancer tissues (Figure 1A).

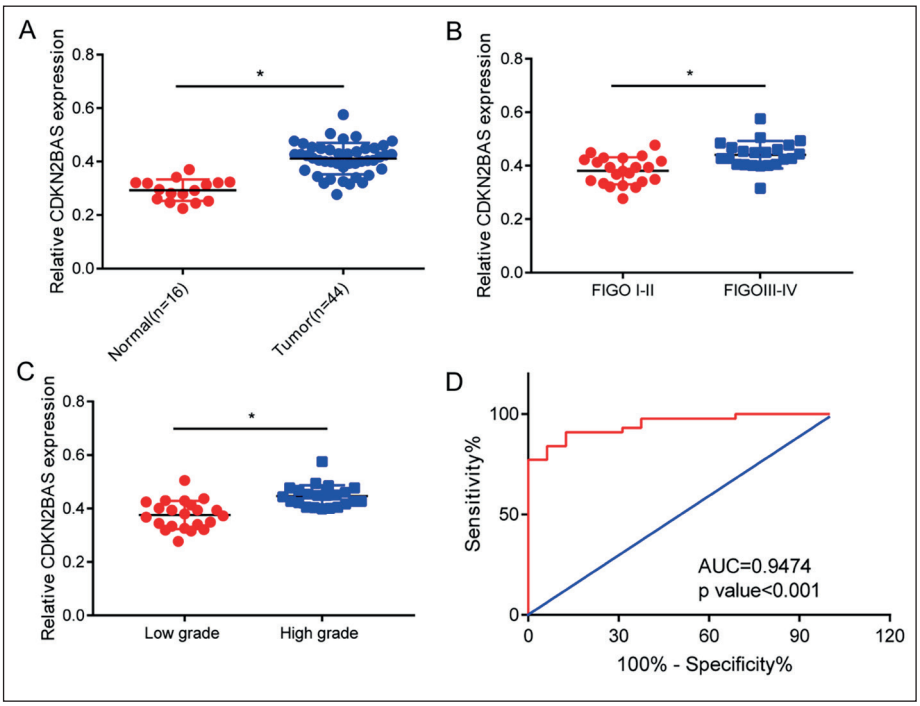

Figure 1. CDKN2BAS was upregulated in ovarian cancer tissues. **A,** Relative levels of CDKN2BAS in ovarian cancer tissues and normal ovarian tissues. **B,** Relative levels of CDKN2BAS in ovarian cancer tissues with FIGO I+II and FIGO III+IV. **C,** Relative levels of CDKN2BAS in ovarian cancer tissues with high and low pathological grade. **D,** ROC curves depicted for assessing the diagnostic potential of CDKN2BAS in ovarian cancer (AUC=0.9474, $p<0.001$, cut-off value=0.3714, sensitivity=90.7%, specificity=89.92%). *$p<0.05$.

According to the FIGO staging, ovarian cancer patients were assigned into FIGO I+II group and FIGO III+IV group. Higher abundance of CDKN2BAS was detected in FIGO III+IV group compared with that in FIGO I+II group (Figure 1B). Similarly, ovarian cancer patients with high pathological grade expressed higher level of CDKN2BAS than those with low grade (Figure 1C). As depicted by ROC curves, the diagnostic potential of CDKN2BAS in ovarian cancer has been identified (AUC=0.9474, $p<0.001$, cut-off value=0.3714, sensitivity=90.7%, specificity=89.92%) (Figure 1D).

Overexpression of CDKN2BAS Promoted Proliferative and Migratory Potentials in Ovarian Cancer

Compared with ovarian epithelial cell line, CDKN2BAS was upregulated in ovarian cancer cell lines (Figure 2A). HEY and SKOV-3 cells were used for establishing *in vitro* CDKN2BAS overexpression models (Figure 2B). The overexpression of CDKN2BAS markedly increased viability in HEY and SKOV-3 cells, suggesting the stimulated proliferative ability (Figure 2C, 2D). Meanwhile, migratory cell number was higher in ovarian cancer cells overexpressing CDKN2BAS than that of controls (Figure 2E). Therefore, CDKN2BAS was able to promote proliferative and migratory potentials in ovarian cancer.

Interaction Between CDKN2BAS and GAS6

It is uncovered that CDKN2BAS was mainly distributed in the cytoplasm of HEY and SKOV-3 cells (Figure 3A, 3B). Moreover, GAS6 was abundantly enriched in anti-CDKN2BAS, verifying the interaction between CDKN2BAS and GAS6 (Figure 3C). In ovarian cancer tissues, GAS6 was markedly upregulated and positively correlated to CDKN2BAS level (Figure 3D, 3E). As expected, GAS6 was upregulated in ovarian cancer cells overexpressing CDKN2BAS (Figure 3F).

CDKN2BAS/GAS6 Axis Was Responsible for Regulating the Progression of Ovarian Cancer

We thereafter explored the potential function of GAS6 in influencing the progression of ovarian cancer. Transfection efficacy of si-GAS6 was first tested in HEY and SKOV-3 cells (Figure 4A). Compared with ovarian cancer cells overexpressing CDKN2BAS, those co-transfected with pcDNA-CDKN2BAS and si-GAS6 presented lower viability and migratory cell number (Figure 4B-4D). It is suggested that the knockdown of GAS6 was able to reverse the regulatory effects of CDKN2BAS on proliferative and migratory potentials in ovarian cancer.

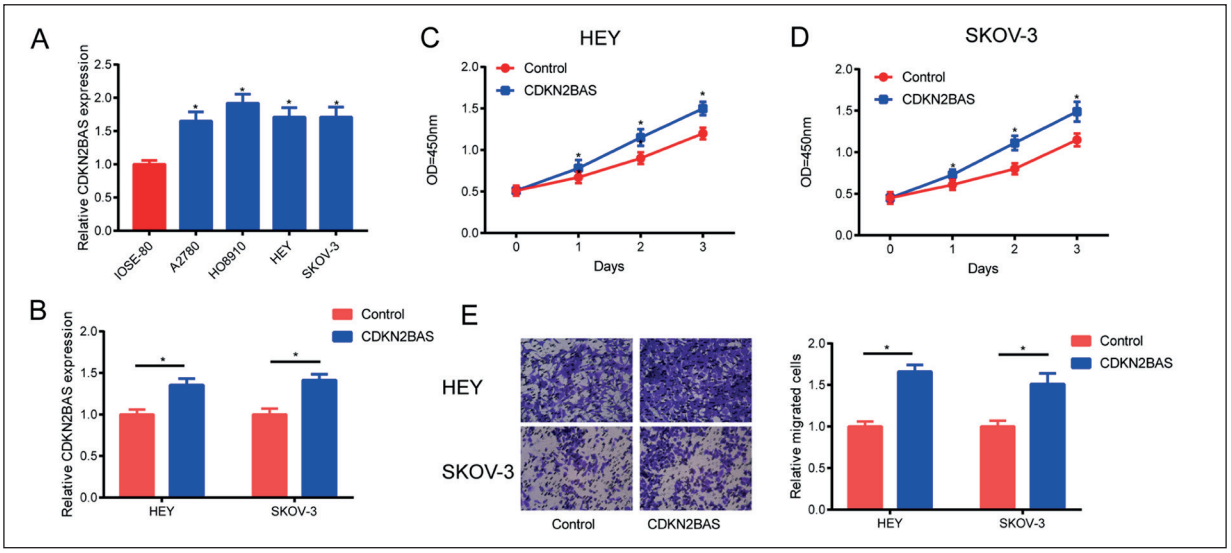

Figure 2. Overexpression of CDKN2BAS promoted proliferative and migratory potentials in ovarian cancer. **A,** Relative levels of CDKN2BAS in ovarian cancer cell lines. **B,** Transfection efficacy of pcDNA-CDKN2BAS in HEY and SKOV-3 cells. **C, D,** Viability in HEY (**C**) and SKOV-3 cells (**D**) transfected with pcDNA-NC or pcDNA-CDKN2BAS at day 0-3. **E,** Migration in HEY and SKOV-3 cells transfected with pcDNA-NC or pcDNA-CDKN2BAS (magnification: 200×) **p*<0.05.

Discussion

Ovarian cancer is a fatal gynecological malignancy, accounting for more than 3% of female tumor cases[15]. It is the fifth most-common reason for cancer death. The mortality of ovarian cancer has not been largely reduced even though therapeutic strategies are improved[16]. Most ovarian cancer patients are observed in the middle or late stage at the first time of diagnosis, and they poorly respond to traditional anti-cancer treatment[17]. Searching effective and specific biomarkers for screening and diagnosing ovarian cancer contributes to improve the clinical outcomes.

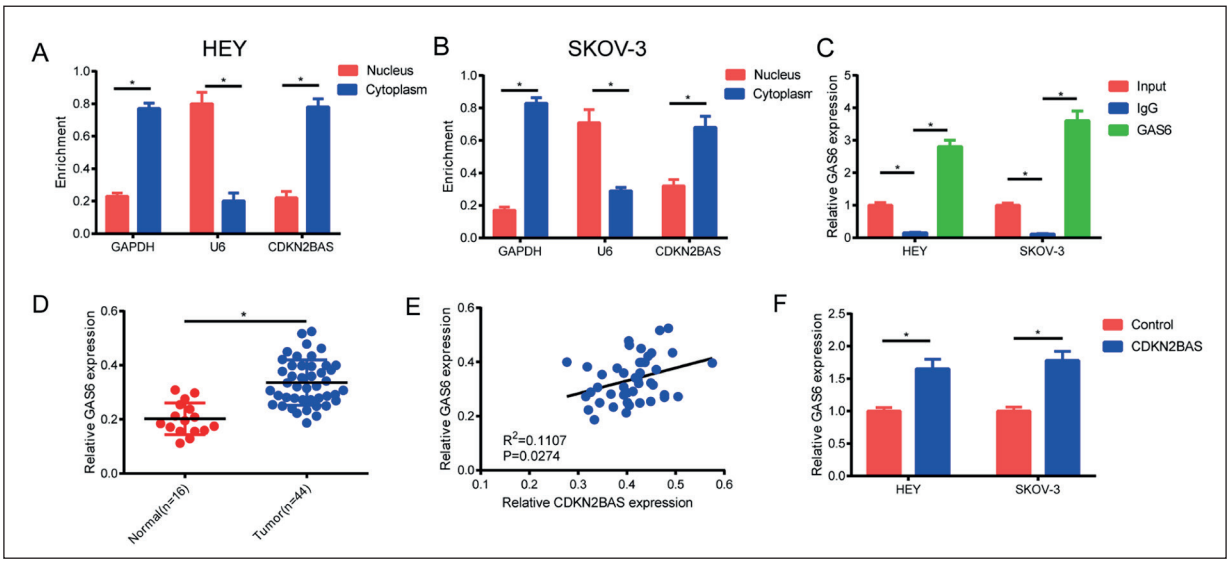

Figure 3. Interaction between CDKN2BAS and GAS6. **A, B,** Subcellular distribution of CDKN2BAS in HEY (**A**) and SKOV-3 cells (**B**). GAPDH and U6 were the internal references of the cytoplasm and nucleus, respectively. **C,** Immunoprecipitant of GAS6 in input, anti-IgG and anti-CDKN2BAS in HEY and SKOV-3 cells. **D,** Relative levels of GAS6 in ovarian cancer tissues and normal ovarian tissues. **E,** A positive correlation between expression levels of CDKN2BAS and GAS6 in ovarian cancer tissues. **F,** Relative level of GAS6 in HEY and SKOV-3 cells transfected with pcDNA-NC or pcDNA-CDKN2BAS. **p*<0.05.

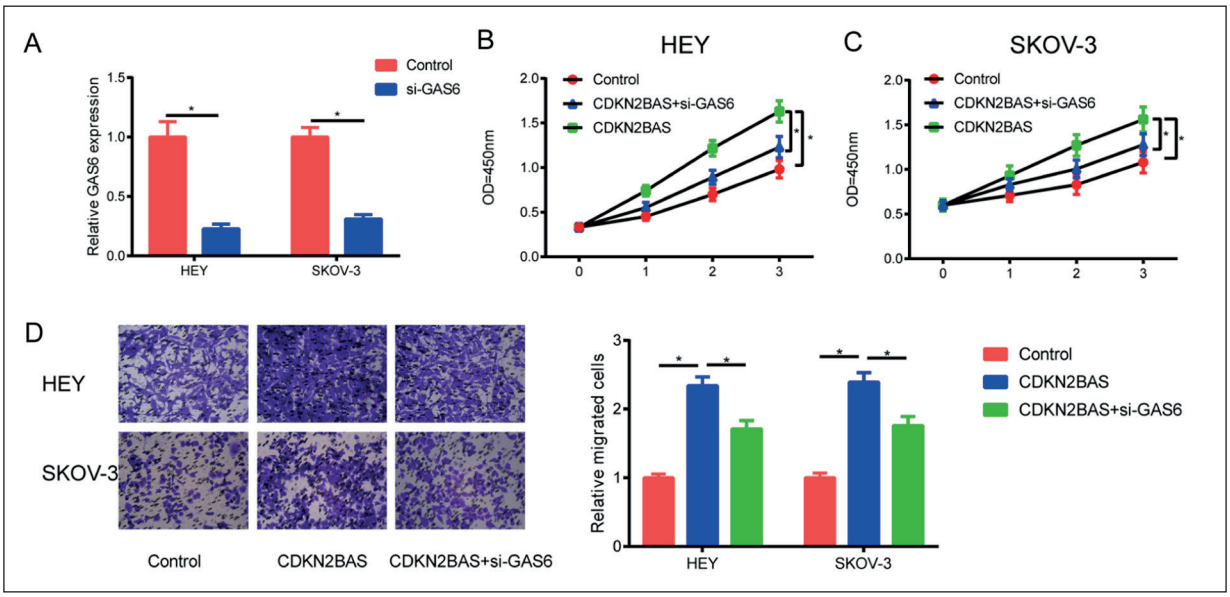

Figure 4. CDKN2BAS/GAS6 axis was responsible for regulating the progression of ovarian cancer. **A,** Transfection efficacy of si-GAS6 in HEY and SKOV-3 cells. **B, C,** Viability in HEY (**B**) and SKOV-3 cells (**C**) transfected with pcDNA-NC, pcDNA-CDKN2BAS or pcDNA-CDKN2BAS+si-GAS6 at day 0-3. **D,** Migration in HEY and SKOV-3 cells transfected with pcDNA-NC, pcDNA-CDKN2BAS or pcDNA-CDKN2BAS+si-GAS6 (magnification: 200×) *p<0.05.

LncRNAs are non-coding RNAs, serving as vital regulators in life activities[18,19]. Abnormally expressed lncRNAs greatly influence the occurrence and progression of tumors, which may be promising tumor biomarkers[20,21]. Differentially expressed lncRNAs have been detected between ovarian cancer samples and normal ones[22,23]. Yong et al[24] demonstrated that lncRNA NEAT1 drives the malignant progression of high-grade serous ovarian cancer. Hence, ovarian cancer-associated lncRNAs can be utilized as diagnostic or therapeutic targets. In our research, CDKN2BAS was detected to be upregulated in ovarian cancer tissues, especially those in advanced FIGO stage or high pathological grade. Furthermore, ROC curves proved that CDKN2BAS may be used as a diagnostic marker for ovarian cancer. *In vitro* studies have indicated the promotive role of CDKN2BAS in regulating proliferative and migratory potentials in ovarian cancer cells.

GAS6 is demonstrated to be linked to diabetes mellitus and vascular complications of diabetes[25,26]. In addition, it also participates in tumor progression[27,28]. Tumor cell metastasis can be triggered by the GAS6/Axl axis through MMP-2-dependent activation of the PI3K/Akt pathway[29]. The overexpression of LINC00565 aggravates the progression of ovarian cancer by targeting GAS6[14]. Our data uncovered that GAS6 was upregulated in ovarian cancer tissues and its level was positively regulated by CDKN2BAS. They were synergistically responsible for the progression of ovarian cancer. As a result, CDKN2BAS/GAS6 axis may be a potential therapeutic target for ovarian cancer.

Conclusions

In summary, lncRNA CDKN2BAS is upregulated in ovarian cancer. By positively interacting with GAS6, CDKN2BAS promotes the proliferative and migratory potentials, thus triggering the progression of ovarian cancer. This study is the first to discover the role of CDKN2BAS in promoting ovarian cancer, which can promote the proliferation and migration of ovarian cancer cells through the regulation of GAS6. However, the specific mechanism of CDKN2BAS in ovarian cancer still needs to be further studied.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Lorusso D, Naldini A, Testa A, D'Agostino G, Scambia G, Ferrandina G. Phase II study of pegylated liposomal doxorubicin in heavily pretreated epithelial ovarian cancer patients. May a new treatment schedule improve toxicity profile? Oncology 2004; 67: 243-249.
2) Eeles RA, Morden JP, Gore M, Mansi J, Glees J, Wenczl M, Williams C, Kitchener H, Osborne R, Guthrie D, Harper P, Bliss JM. Adjuvant hormone therapy may improve survival in epithelial ovarian cancer: results of the AHT randomized trial. J Clin Oncol 2015; 33: 4138-4144.
3) Lennox KA, Behlke MA. Cellular localization of long non-coding RNAs affects silencing by RNAi more than by antisense oligonucleotides. Nucleic Acids Res 2016; 44: 863-877.
4) Yang L, Lin C, Jin C, Yang JC, Tanasa B, Li W, Merkurjev D, Ohgi KA, Meng D, Zhang J, Evans CP, Rosenfeld MG. LncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. Nature 2013; 500: 598-602.
5) Zhou M, Sun Y, Sun Y, Xu W, Zhang Z, Zhao H, Zhong Z, Sun J. Comprehensive analysis of lncRNA expression profiles reveals a novel lncRNA signature to discriminate nonequivalent outcomes in patients with ovarian cancer. Oncotarget 2016; 7: 32433-32448.
6) Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-463.
7) Cunnington MS, Santibanez KM, Mayosi BM, Burn J, Keavney B. Chromosome 9p21 SNPs associated with multiple disease phenotypes correlate with ANRIL expression. PLoS Genet 2010; 6: e1000899.
8) Rivandi M, Khorrami MS, Fiuji H, Shahidsales S, Hasanzadeh M, Jazayeri MH, Hassanian SM, Ferns GA, Saghafi N, Avan A. The 9p21 locus: a potential therapeutic target and prognostic marker in breast cancer. J Cell Physiol 2018; 233: 5170-5179.
9) Adel FM, Lavebratt C, Schuz J, Roosli M, Tynes T, Grotzer MA, Johansen C, Kuehni CE, Lannering B, Prochazka M, Schmidt LS, Feychting M. CCDC26, CDKN2BAS, RTEL1 and TERT polymorphisms in pediatric brain tumor susceptibility. Carcinogenesis 2015; 36: 876-882.
10) Chen YD, Zhang N, Qiu XG, Yuan J, Yang M. LncRNA CDKN2BAS rs2157719 genetic variant contributes to medulloblastoma predisposition. J Gene Med 2018; 20. doi: 10.1002/jgm.3000.
11) Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. Cell 1988; 54: 787-793.
12) Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. Mol Cell Biol 1993; 13: 4976-4985.
13) Sun W, Fujimoto J, Tamaya T. Coexpression of Gas6/Axl in human ovarian cancers. Oncology 2004; 66: 450-457.
14) Gong M, Luo C, Meng H, Li S, Nie S, Jiang Y, Wan Y, Li H, Cheng W. Upregulated LINC00565 accelerates ovarian cancer progression by targeting GAS6. Onco Targets Ther 2019; 12: 10011-10022.
15) Soletormos G, Duffy MJ, Othman AHS, Verheijen RH, Tholander B, Bast RJ, Gaarenstroom KN, Sturgeon CM, Bonfrer JM, Petersen PH, Troonen H, CarloTorre G, Kanty KJ, Tuxen MK, Molina R. Clinical use of cancer biomarkers in epithelial ovarian cancer: updated guidelines from the European Group on Tumor Markers. Int J Gynecol Cancer 2016; 26: 43-51.
16) Cannioto RA, LaMonte MJ, Kelemen LE, Risch HA, Eng KH, Minlikeeva AN, Hong CC, Szender JB, Sucheston-Campbell L, Joseph JM, Berchuck A, Chang-Claude J, Cramer DW, DeFazio A, Diergaarde B, Dork T, Doherty JA, Edwards RP, Fridley BL, Friel G, Goode EL, Goodman MT, Hillemanns P, Hogdall E, Hosono S, Kelley JL, Kjaer SK, Klapdor R, Matsuo K, Odunsi K, Nagle CM, Olsen CM, Paddock LE, Pearce CL, Pike MC, Rossing MA, Schmalfeldt B, Segal BH, Szamreta EA, Thompson PJ, Tseng CC, Vierkant R, Schildkraut JM, Wentzensen N, Wicklund KG, Winham SJ, Wu AH, Modugno F, Ness RB, Jensen A, Webb PM, Terry K, Bandera EV, Moysich KB. Recreational physical inactivity and mortality in women with invasive epithelial ovarian cancer: evidence from the Ovarian Cancer Association Consortium. Br J Cancer 2016; 115: 95-101.
17) Ramalingam P. Morphologic, immunophenotypic, and molecular features of epithelial ovarian cancer. Oncology (Williston Park) 2016; 30: 166-176.
18) Fan YH, Ji CX, Xu B, Fan HY, Cheng ZJ, Zhu XG. Long noncoding RNA activated by TGF-beta in human cancers: a meta-analysis. Clin Chim Acta 2017; 468: 10-16.
19) Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttman M, Lander ES. Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016; 539: 452-455.
20) Zou ZW, Ma C, Medoro L, Chen L, Wang B, Gupta R, Liu T, Yang XZ, Chen TT, Wang RZ, Zhang WJ, Li PD. LncRNA ANRIL is up-regulated in nasopharyngeal carcinoma and promotes the cancer progression via increasing proliferation, reprograming cell glucose metabolism and inducing side-population stem-like cancer cells. Oncotarget 2016; 7: 61741-61754.
21) Zang W, Wang T, Huang J, Li M, Wang Y, Du Y, Chen X, Zhao G. Long noncoding RNA PEG10 regulates proliferation and invasion of esophageal cancer cells. Cancer Gene Ther 2015; 22: 138-144.
22) Chai Y, Liu J, Zhang Z, Liu L. HuR-regulated lncRNA NEAT1 stability in tumorigenesis and progression of ovarian cancer. Cancer Med 2016; 5: 1588-1598.

23) Zhang Z, Cheng J, Wu Y, Qiu J, Sun Y, Tong X. LncRNA HOTAIR controls the expression of Rab22a by sponging miR-373 in ovarian cancer. Mol Med Rep 2016; 14: 2465-2472.

24) Yong W, Yu D, Jun Z, Yachen D, Weiwei W, Midie X, Xingzhu J, Xiaohua W. Long noncoding RNA NEAT1, regulated by LIN28B, promotes cell proliferation and migration through sponging miR-506 in high-grade serous ovarian cancer. Cell Death Dis 2018; 9: 861.

25) Angelillo-Scherrer A, de Frutos P, Aparicio C, Melis E, Savi P, Lupu F, Arnout J, Dewerchin M, Hoylaerts M, Herbert J, Collen D, Dahlback B, Carmeliet P. Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. Nat Med 2001; 7: 215-221.

26) Lee CH, Chu NF, Shieh YS, Hung YJ. The growth arrest-specific 6 (Gas6) gene polymorphism c.834+7G>A is associated with type 2 diabetes. Diabetes Res Clin Pract 2012; 95: 201-206.

27) Kariolis MS, Miao YR, Diep A, Nash SE, Olcina MM, Jiang D, Jones DN, Kapur S, Mathews II, Koong AC, Rankin EB, Cochran JR, Giaccia AJ. Inhibition of the GAS6/AXL pathway augments the efficacy of chemotherapies. J Clin Invest 2017; 127: 183-198.

28) Shiozawa Y, Pedersen EA, Patel LR, Ziegler AM, Havens AM, Jung Y, Wang J, Zalucha S, Loberg RD, Pienta KJ, Taichman RS. GAS6/AXL axis regulates prostate cancer invasion, proliferation, and survival in the bone marrow niche. Neoplasia 2010; 12: 116-127.

29) Rankin EB, Fuh KC, Taylor TE, Krieg AJ, Musser M, Yuan J, Wei K, Kuo CJ, Longacre TA, Giaccia AJ. AXL is an essential factor and therapeutic target for metastatic ovarian cancer. Cancer Res 2010; 70: 7570-7579.