Am J Transl Res 2017;9(9):4094-4103
www.ajtr.org /ISSN:1943-8141/AJTR0049706

Original Article

LncRNA EWSAT1 promotes ovarian cancer progression through targeting miR-330-5p expression

Xin Fu[1], Li Zhang[1], Li Dan[1], Ke Wang[1], Yue Xu[2]

[1]*Department of Gynecology Cancer, National Clinical Research Center for Cancer, Tianjin Key Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin 300060, China;* [2]*Laboratory of Cancer Cell Biology, National Clinical Research Center for Cancer, Tianjin Key Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin 300060, China*

Received January 25, 2017; Accepted May 4, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: Ovarian cancer ranks as the fifth common tumour in women and is the leading cause of cancer-related death among the women. Long non-coding RNAs (lncRNAs) play crucial roles in the development of tumors. However, the expression and the role of EWSAT1 in ovarian tumor are still unknown. In our study, we found the expression of EWSAT1 was upregulated in ovarian cancer cell lines and samples. Ecoptic expression of EWSAT1 suppressed miR-330-5p expression in ovarian cancer cell. In addition, the expression of miR-330-5p was downregulated in ovarian cancer cell lines and samples. Moreover, the expression of miR-330-5p was negatively related with the expression of EWSAT1 in ovarian cancer samples. Furthermore, we identified Pdia3 was a direct target gene of miR-330-5p. Ecoptic expression of miR-330-5p suppressed Pdia3 expression and overexpression of EWSAT1 promoted Pdia3 expression in ovarian cancer cell. In addition, Ecoptic expression of miR-330-5p promoted ovarian cancer cell proliferation, colony formation and invasion. Overexpression of EWSAT1 increased ovarian cancer cell proliferation, colony formation and invasion through targeting miR-330-5p. These data suggested that EWSAT1 might act as an oncogene in the development of ovarian cancer partly through inhibiting miR-330-5p expression.

Keywords: Ovarian cancer, long non-coding RNAs, EWSAT1, miR-330-5p

Introduction

Ovarian cancer ranks as the fifth common tumour in women and is the leading cause of cancer-related death among women [1-4]. Many ovarian cancer patients are diagnosed at stage IV or III, which lead to high mortality rate [5-7]. Despite the advances in the treatment such as surgical technique, chemotherapy regimens and radiotherapy, the five-year survival rate is still not satisfied [8-10]. It is imperative to elucidate the molecular mechanism of this disease and identify the biomarkers for treatment of ovarian cancer.

Long non-coding RNAs (lncRNAs) are a new class of ncRNAs that are greater than 200 nucleotides with no protein-coding function [11-14]. Recent data have revealed that lncRNAs play an essential role in diverse cellular functions including cell development, proliferation, differentiation, apoptosis and invasion [15-18]. A number of lncRNAs are found to be deregulated in various tumors such as colorectal carcinoma, osteosarcoma, hepatocellular carcinoma, gastric cancer, bladder cancer and lung cancer [19-24]. Recently, lncRNA Ewing sarcoma-associated transcript 1 (EWSAT1) was found to be upregulated in Ewing sarcoma and nasopharyngeal carcinoma [25, 26]. However, the expression and the role of EWSAT1 in ovarian tumor are still unknown.

In our study, we tried to study the expression and the role of EWSAT1 in ovarian tumor. We indicated that the expression of EWSAT1 was upregulated in ovarian cancer cell lines and samples. Overexpression of EWSAT1 promoted ovarian cancer cell proliferation, colony formation and invasion by targeting the miR-330-5p.

Materials and methods

Tissue samples

Primary ovarian tumor tissues and matched adjacent normal samples were obtained from Tianjin Medical University Cancer Institute and

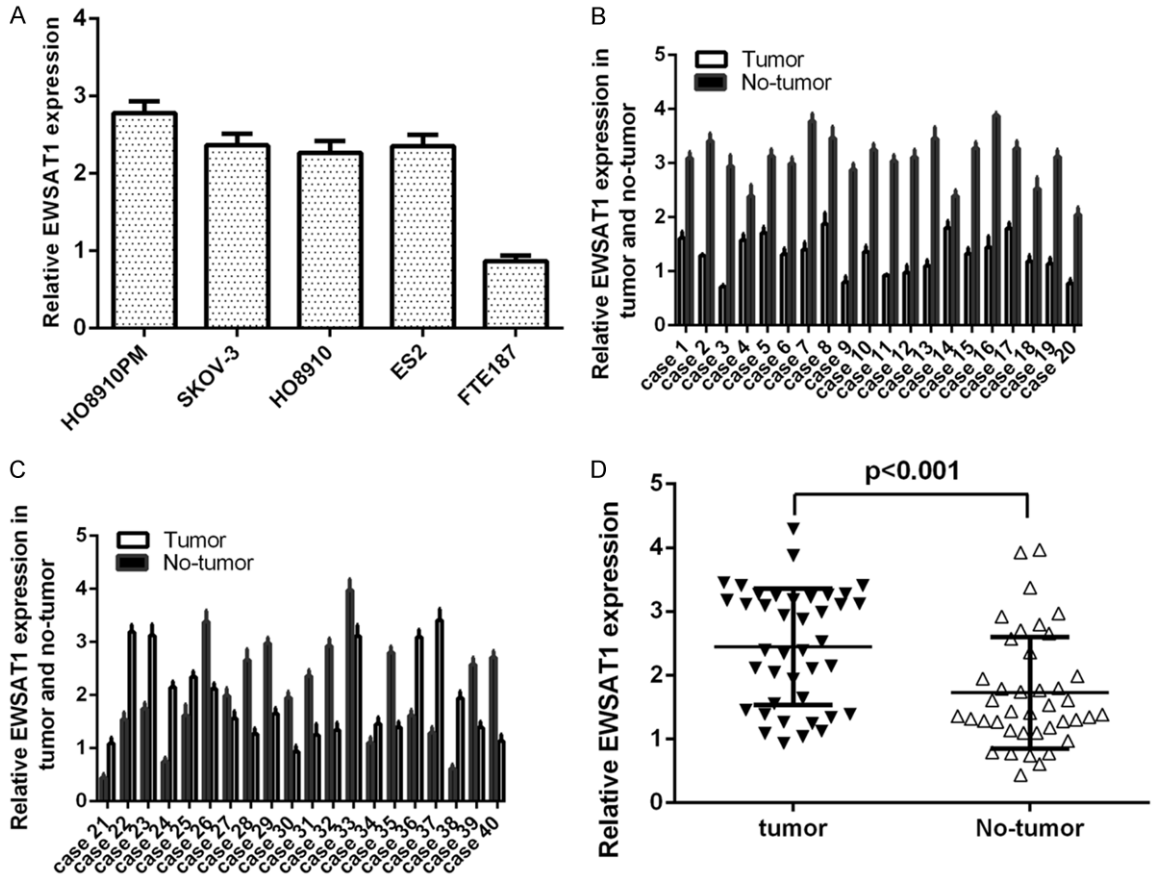

Figure 1. EWSAT1 expression was upregulated in the ovarian cancer cell lines and tissues. A: The EWSAT1 expression in the 4 ovarian cancer cell lines (SKOV-3, HO8910, HO8910PM and, ES2) and immortalized normal fallopian tube epithelial cell line (FTE187) was analyzed by qRT-PCR. B, C: The expression of EWSAT1 in the 40 pairs' ovarian cancer samples and non-tumorous tissues was shown. D: The expression level of EWSAT1 was higher in the ovarian tumor samples compared with that in the adjacent normal samples. ***P<0.001.

Hospital. All patients gave the written informed consent and this study was approved by the institutional ethics review board of Tianjin Medical University Cancer Institute and Hospital.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from clinical tissues and cell lines with TRIzol reagent (Invitrogen, USA) following to protocol. Quantitative Real-time PCR (qRT-PCR) was performed to determine the expression of lncRNA and mRNA using SYBR green (Takara, Dalian, Japan) on the 7500 Fast System. The primer for EWSAT1 is as follows: forward, GTGTCTGGCAAGGAACACTA, GGTGGAGAAGAGGGACAATAAG. The primer for GAPDH follows: forward, TTGGTATCGTGGAAGGACTCA, reverse, TGTCATCATATTTGGCAGGTT. The U6 and GAPDH were used as endogenous controls for EWSAT1 and Piad3, respectively.

Cell culture and plasmids transfection

An immortalized normal fallopian tube epithelial cell line (FTE187) and 5 ovarian cancer cell lines (SKOV-3, HO8910, HO8910PM, ES2 and HG-SOC) were obtained from Chinese Academy of Sciences (Shanghai, China). Cells were grown in RPMI-1640 medium with fetal bovine serum (FBS). EWSAT1 and control plasmids, miR-330-5p mimic and scramble mimics were obtained from GenePharma Company (Shanghai, PR China) and were tranfected to cells by using Lipofectamine 2000 (Invitrogen, USA).

Cell proliferation, colony formation and migration assays

MTT assay was performed to estimate the cell proliferation. Cells were cultured in a 96 well culture plate for 24, 48 and 72 hours. Absorbances were determined at the wave-

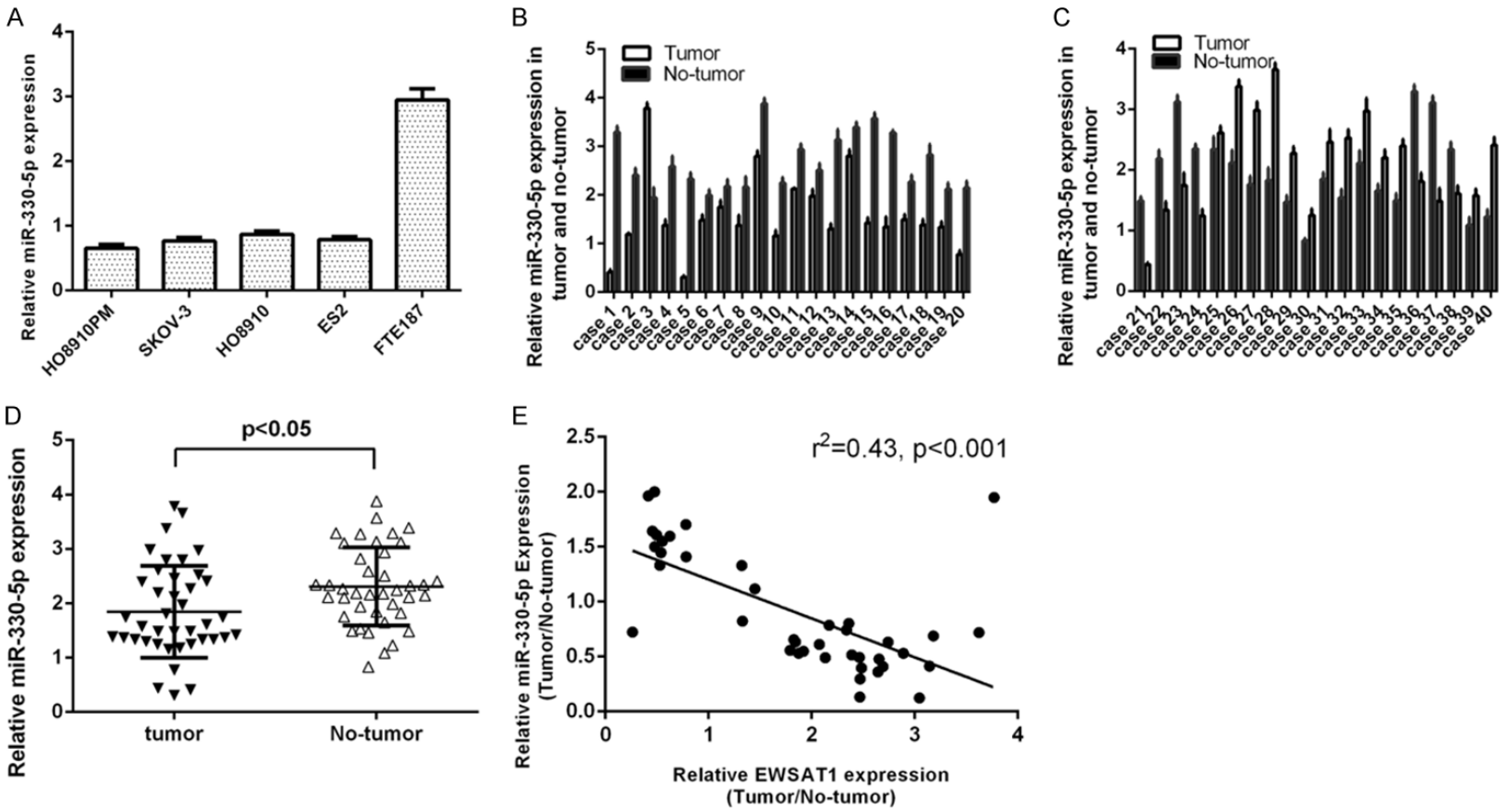

Figure 2. miR-330-5p expression was downregulated in the ovarian cancer cell lines and tissues. A: The miR-330-5p expression in the 4 ovarian cancer cell lines (SKOV-3, HO8910, HO8910PM and, ES2) and FTE187 was analyzed by qRT-PCR. B, C: The expression of miR-330-5p in the 40 pairs' ovarian cancer samples and non-tumorous tissues was shown. D: The expression level of miR-330-5p was lower in the ovarian tumor samples compared with that in the adjacent normal samples. E: The expression of miR-330-5p was negatively related with the expression of EWSAT1 in the ovarian cancer samples. ***P<0.001.

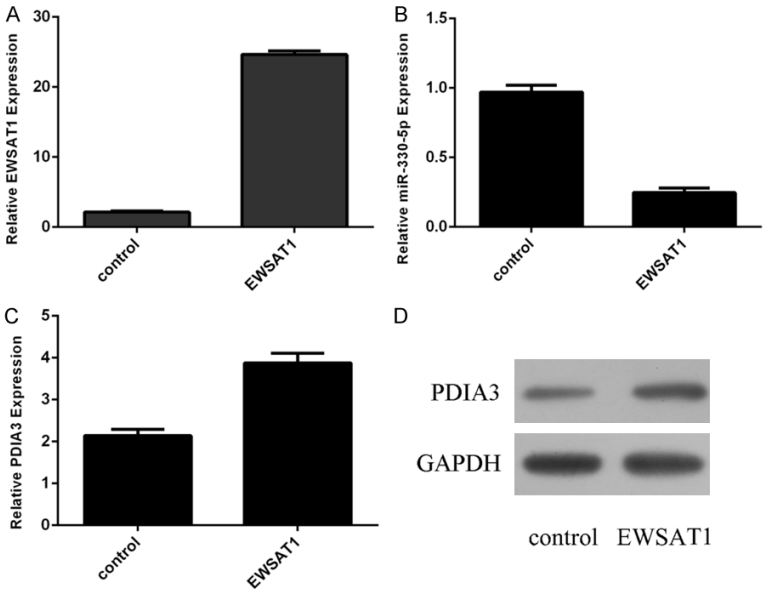

Figure 3. Ectopic expression of EWSAT1 suppressed the miR-330-5p expression. A: The EWSAT1 expression in the HO8910PM cell was analyzed by qRT-PCR. B: Ecoptic expression of EWSAT1 suppressed the miR-330-5p expression in the HO8910PM cell. C: Overexpression of EWSAT1 promoted the Pdia3 expression. D: The protein expression of Pdia3 was analyzed by western blot. *P<0.05, **P<0.01 and ***P<0.001.

length of 490 nM by using a spectrophotometer (Thermo, Shimadzu, Japan). To estimate the cell colony formation, the transfected cells were seeded in the 12-well plate. After 2 weeks, the cell colonies were stained by crystal violet and counted. For cell migration assay, wound-healing was performed. Cells were seeded in the 6-well plate and the cell layer was created by the sterile plastic tip. Photo of the cell layer was taken using a microscope.

Western blotting

Protein was isolated from cultured cells or human samples using the Protein Extraction Kit (KeyGen, China). Protein concentration was measured by a BCA Assay Kit (KeyGen, China). Total protein was separated through 10% SDS-PAGE and tranferred to membrane (Millipore, MA, USA). The membrane was blocked with 5% milk and then incubated with antibodies (Pdia3 and GAPDH) at 4°C overnight. Blot was treated with the HRP conjugated antibody and the signal was measured by ECL Kit (Pierce, IL, USA).

Statistical analyses

All the results were presented as mean ± standard deviation (SD). All statistical assays were measured by using SPSS 17.0 software (SPSS Inc., Chicago, USA). Student's t test was performed to test the significance between 2 groups and the significance between three or more groups was determined by one-way ANOVA. P<0.05 was considered to be statistically significant.

Results

EWSAT1 expression was upregulated in ovarian cancer cell lines and tissues

EWSAT1 expression was lower in 4 ovarian cancer cell lines (SKOV-3, HO8910, HO8910PM and, ES2) than in immortalized normal fallopian tube epithelial cell line (FTE187) (**Figure 1A**). The expression of EWSAT1 in 40 pairs of ovarian cancer samples and non-tumorous tissues was shown in **Figure 1B** and **1C**. The expression level of EWSAT1 was higher in ovarian tumor samples compared with that in the adjacent normal samples (**Figure 1D**).

miR-330-5p expression was downregulated in ovarian cancer cell lines and tissues

The miR-330-5p expression was higher in 4 ovarian cancer cell lines (SKOV-3, HO8910, HO8910PM and, ES2) than in immortalized normal fallopian tube epithelial cell line (FTE187) (**Figure 2A**). The expression of miR-330-5p in 40 pairs of ovarian cancer samples and non-tumorous tissues was shown in the **Figure 2B** and **2C**. The expression level of miR-330-5p was lower in ovarian tumor samples compared with that in the adjacent normal samples (**Figure 2D**). Moreover, the expression of miR-330-5p was negatively related with the expression of EWSAT1 in ovarian cancer samples (**Figure 2E**).

Ectopic expression of EWSAT1 suppressed the miR-330-5p expression

qRT-PCR assay showed that EWSAT1 expression was significantly upregulated in the HO-

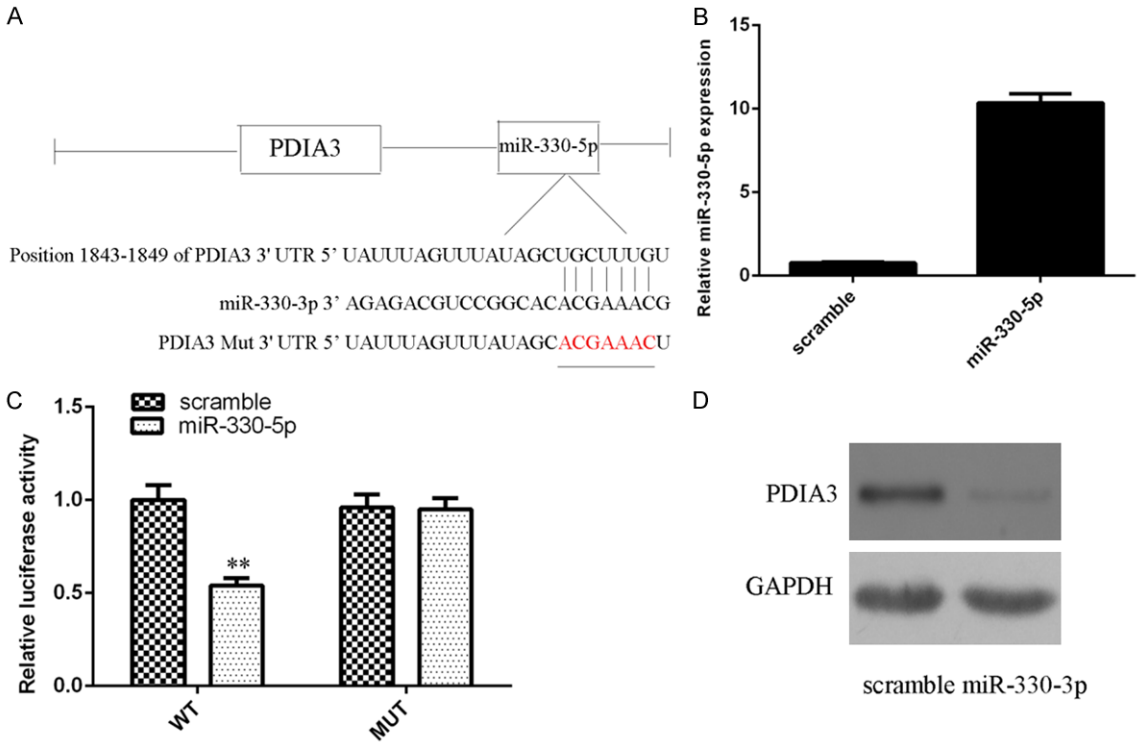

Figure 4. Pdia3 was a direct target gene of miR-330-5p. A: TargetScan system was used to find the potential target gene of miR-330-5 and the potential 3'UTR binding site of Pdia3 of miR-330-5p. B: The miR-330-5p expression was measured in the HO8910PM cell by qRT-PCR. C: Overexpression of miR-330-5p decreased renilla luciferase activity of the WT Pdia3 3'UTR but not the mutant vector (Mut Pdia3 3'UTR). D: Overexpression of miR-330-5p suppressed the Pdia3 expression. **P<0.01.

8910PM cell which was treated with pcDNA-EWSAT1 (**Figure 3A**). Ecoptic expression of EWSAT1 suppressed miR-330-5p expression. In addition, overexpression of EWSAT1 promoted the Pdia3 mRNA and protein expression (**Figure 3C** and **3D**).

Pdia3 was a direct target gene of miR-330-5p

We used the TargetScan system to find the potential target gene of miR-330-5 and the potential 3'-UTR binding site of Pdia3 of miR-330-5p was shown in **Figure 4A**. qRT-PCR assay showed that the miR-330-5p expression was significantly upregulated in the HO8910PM cell that was treated with miR-330-5p mimic (**Figure 4B**). As shown in the **Figure 4C**, overexpression of miR-330-5p decreased renilla luciferase activity of the WT Pdia3 3'UTR but not the mutant vector (Mut Pdia3 3'UTR). Overexpression of miR-330-5p suppressed the Pdia3 expression (**Figure 4D**).

Ecoptic expression of EWSAT1 promoted ovarian cancer cell proliferation, colony formation and invasion

Ecoptic expression of EWSAT1 increased the cell proliferation in ovarian cancer line HO8910PM (**Figure 5A**) and SKOV-3 (**Figure 5B**). Elevated expression of EWSAT1 promoted cell colony formation in HO8910PM and SKOV-3 (**Figure 5C**). Moreover, overexpression of EWSAT1 increased cell invasion in HO8910PM and SKOV-3 (**Figure 5D**).

EWSAT1 promoted ovarian cancer cell proliferation, colony formation and invasion by inhibiting the miR-330-5p expression

To explore whether miR-330-5p involved in the process of EWSAT1 induced the HO8910PM, we rescued the miR-330-5p expression in the EWSAT1-overexpressing HO8910PM cell. Ectopic expression of miR-330-5p prevented the HO8910PM cell proliferation induced by over-

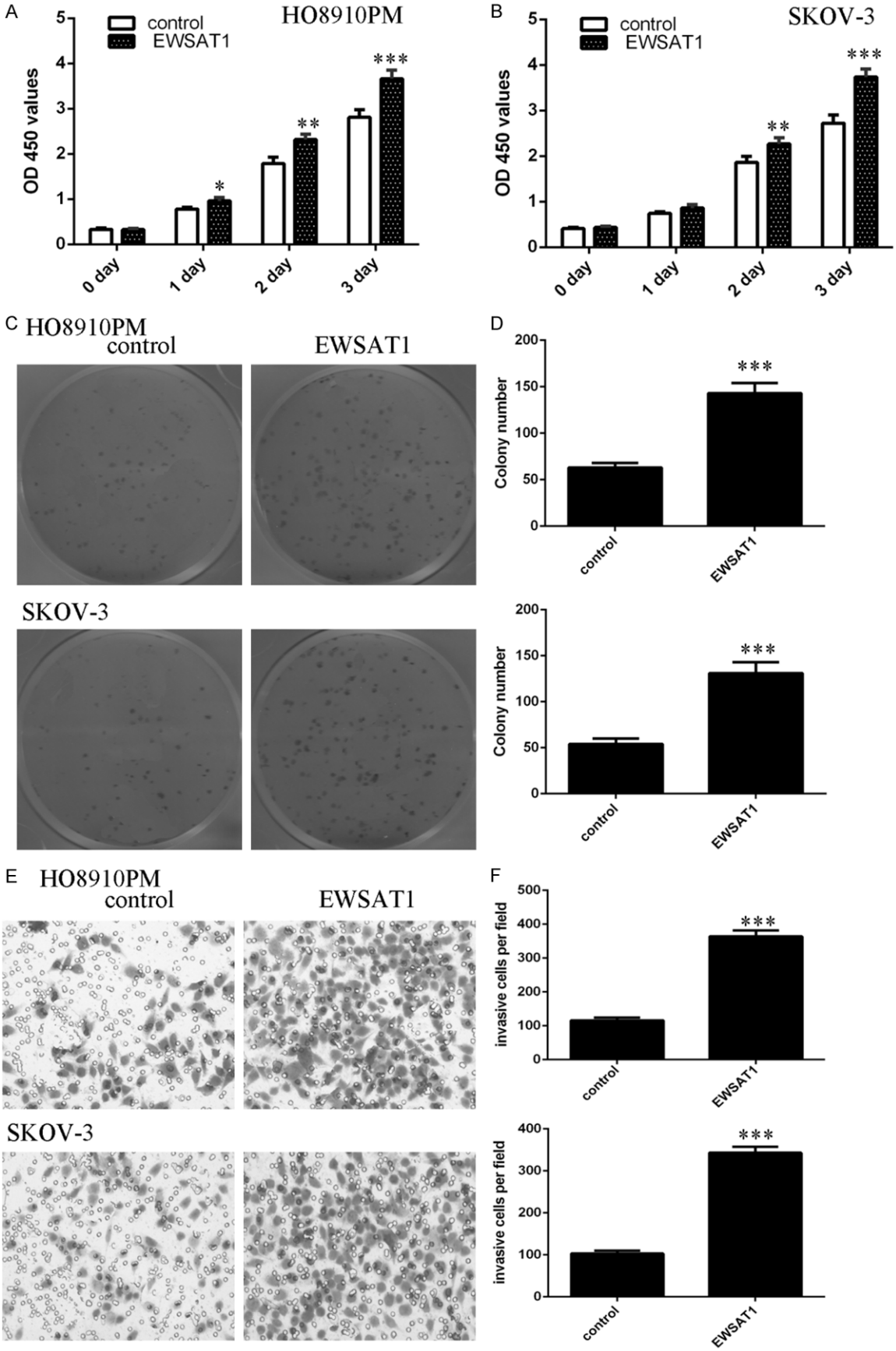
A
HO8910PM
control
EWSAT1
OD 450 values
0 day
1 day
2 day
3 day
B
SKOV-3
C
HO8910PM
control
EWSAT1
SKOV-3
D
Colony number
E
F
invasive cells per field

Figure 5. Ecoptic expression of EWSAT1 promoted the ovarian cancer cell proliferation, colony formation and invasion. A: Ecoptic expression of EWSAT1 increased the HO8910PM cell proliferation. B: Ecoptic expression of EWSAT1 promoted the SKOV-3 cell proliferation. C: Elevated expression of EWSAT1 promoted the HO8910PM and SKOV-3 cell colony formation. D: The relative colony number was shown. E: Overexpression of EWSAT1 increased the HO8910PM and SKOV-3 cell invasion. F: The relative invasive cells were shown. *P<0.05, **P<0.01 and ***P<0.001.

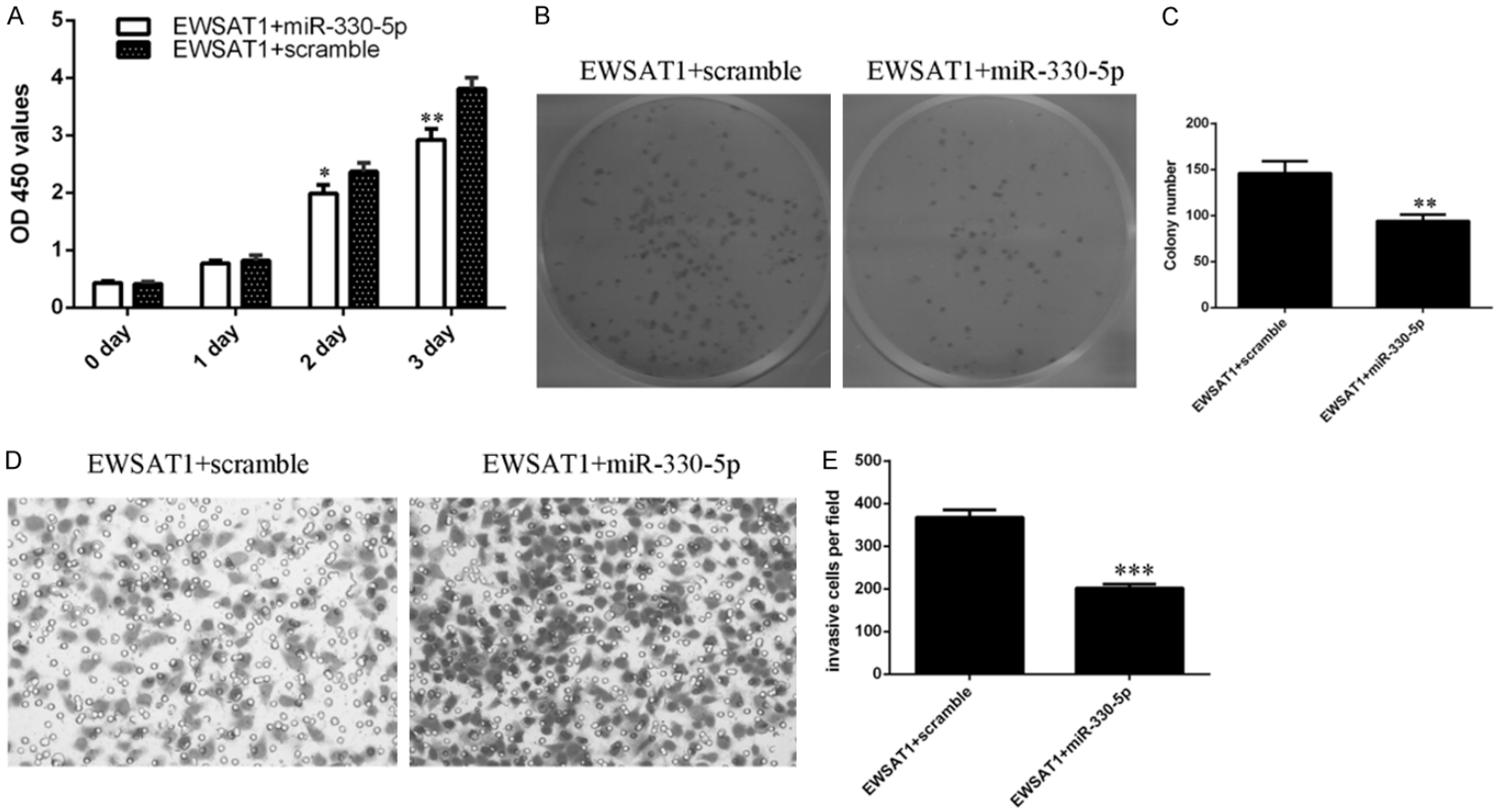

Figure 6. EWSAT1 promoted the ovarian cancer cell proliferation, colony formation and invasion by inhibiting the miR-330-5p expression. A: The cell proliferation was measured by MTT assay. B: Elevated expression of miR-330-5p decreased the HO8910PM cell colony formation induced by overexpression of EWSAT1. C: The relative colony number was shown. D: Overexpression of miR-330-5p prevented the HO8910PM cell invasion induced by overexpression of EWSAT1. E: The relative invasive cells were shown. *P<0.05, **P<0.01 and ***P<0.001.

expression of EWSAT1 (**Figure 6A**). Elevated expression of miR-330-5p decreased the HO8910PM cell colony formation induced by overexpression of EWSAT1 (**Figure 6B**). In addition, overexpression of miR-330-5p prevented the HO8910PM cell invasion induced by overexpression of EWSAT1 (**Figure 6C**).

Discussion

In our study, we found that the expression of EWSAT1 was upregulated in ovarian cancer cell lines and samples. Ecoptic expression of EWSAT1 suppressed miR-330-5p expression in ovarian cancer cell. We demonstrated that the expression of miR-330-5p was downregulated in ovarian cancer cell lines and samples. Moreover, the expression of miR-330-5p was negatively related with the expression of EWSAT1 in ovarian cancer samples. Furthermore, we identified that Pdia3 was a direct target gene of miR-330-5p. Ecoptic expression of miR-330-5p suppressed Pdia3 expression and overexpression of EWSAT1 promoted the Pdia3 expression in ovarian cancer cell. In addition, ecoptic expression of miR-330-5p promoted ovarian cancer cell proliferation, colony formation and invasion. Overexpression of EWSAT1 increased ovarian cancer cell proliferation, colony formation and invasion through targeting miR-330-5p. These data suggested that EWSAT1 might act as an oncogene in the development of ovarian cancer partly through inhibiting miR-330-5p expression.

LncRNA EWSAT1 is located on the chromosome 15 between two protein-coding genes, GLCE and NOX5. Marques Howarth et al. [25] firstly reported that EWSAT1 expression level was upregulated in Ewing sarcoma. Inhibited expression of EWSAT1 suppressed the Ewing sarcoma cell proliferation and colony formation. Song et al. [26] demonstrated that EWSAT1 expression level was upregulated in nasopharyngeal carcinoma cell lines and samples and higher expression of EWSAT1 was associated with poorer survival time. Overexpression of EWSAT1 increased the nasopharyngeal carcinoma cell proliferation. Ectopic expression of EWSAT1 suppressed the miR-326/330-5p expression and increased the cyclin D1 expression, which was the target gene of miR-326/330-5p. Sun et al. [27] showed that overexpression of EWSAT1 promoted osteosarcoma cell proliferation, migration, and invasion. Moreover, ectopic expression of EWSAT1 enhanced the lncRNA MEG3 expression. Finally, they found that EWSAT1 promoted osteosarcoma cell proliferation and metastasis by regulation of MEG3 expression. However, the role of EWSAT1 in ovarian cancer is still unknown. In this study, we firstly measured the expression of EWSAT1 in ovarian cancer cell lines and tissues. We found that EWSAT1 expression was lower in the 4 ovarian cancer cell lines (SKOV-3, HO8910, HO8910PM and, ES2) than in immortalized normal fallopian tube epithelial cell line (FTE187) (**Figure 1A**). The expression level of EWSAT1 was higher in ovarian tumor samples compared with that in the adjacent normal samples. Moreover, ecoptic expression of EWSAT1 promoted the ovarian cancer cell proliferation, colony formation and invasion. These results suggested that EWSAT1 acted as an oncogene in ovarian cancer.

In line with previous study, we also demonstrated that ecoptic expression of EWSAT1 suppressed miR-330-5p expression in ovarian cancer cell. Previous studies demonstrated that miR-330-5p played important roles in the development and progression of tumors. For example, Kim et al. showed that overexpression of miR-330-5p suppressed the keratinocytes proliferation and migration through targeting Pdia3. Su et al. demonstrated miR-330-5p expression was downregulated in cutaneous malignant melanoma cell lines and samples. Ecoptic expression of miR-330-5p suppressed the cutaneous malignant melanoma cell proliferation and migration by inhibiting the expression of TYR and PDIA3. In our study, we demonstrated that the expression of miR-330-5p was downregulated in ovarian cancer cell lines and samples. In addition, the expression of miR-330-5p was negatively related with the expression of EWSAT1 in ovarian cancer samples. We also identified that Pdia3 was a direct target gene of miR-330-5p in ovarian cancer cell. Elevated expression of miR-330-5p suppressed Pdia3 expression and overexpression of EWSAT1 promoted Pdia3 expression in ovarian cancer cell. Moreover, overexpression EWSAT1 increased ovarian cancer cell proliferation, colony formation and invasion through targeting miR-330-5p.

In conclusion, we indicated that the expression of EWSAT1 was upregulated in ovarian cancer

cell lines and samples. Overexpression of EWSAT1 promoted ovarian cancer cell proliferation, colony formation and invasion by targeting miR-330-5p. These data suggested that EWSAT1 might act as an oncogene in the development of ovarian cancer partly through inhibiting miR-330-5p expression.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No. 81301895).

Disclosure of conflict of interest

None.

Address correspondence to: Xin Fu, Department of Gynecology Cancer, National Clinical Research Center for Cancer, Tianjin Key Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin 300060, China. E-mail: igrer1@163.com

References

[1] Wang L, He J, Xu H, Xu L and Li N. MiR-143 targets CTGF and exerts tumor-suppressing functions in epithelial ovarian cancer. Am J Transl Res 2016; 8: 2716-2726.

[2] Sun TY, Xie HJ, He H, Li Z and Kong LF. miR-26a inhibits the proliferation of ovarian cancer cells via regulating CDC6 expression. Am J Transl Res 2016; 8: 1037-1046.

[3] Chen W, Huang L, Hao C, Zeng W, Luo X, Li X, Zhou L, Jiang S, Chen Z and He Y. MicroRNA-155 promotes apoptosis in SKOV3, A2780, and primary cultured ovarian cancer cells. Tumour Biol 2016; 37: 9289-99.

[4] Fu X, Cui Y, Yang S, Xu Y and Zhang Z. MicroRNA-613 inhibited ovarian cancer cell proliferation and invasion by regulating KRAS. Tumour Biol 2016; 37: 6477-6483.

[5] Zuberi M, Khan I, Gandhi G, Ray PC and Saxena A. The conglomeration of diagnostic, prognostic and therapeutic potential of serum miR-199a and its association with clinicopathological features in epithelial ovarian cancer. Tumour Biol 2016; 37: 11259-66.

[6] Zuberi M, Khan I, Mir R, Gandhi G, Ray PC and Saxena A. Utility of serum miR-125b as a diagnostic and prognostic indicator and its alliance with a panel of tumor suppressor genes in epithelial ovarian cancer. PLoS One 2016; 11: e0153902.

[7] Dong R, Liu X, Zhang Q, Jiang Z, Li Y, Wei Y, Yang Q, Liu J, Wei JJ, Shao C, Liu Z and Kong B. miR-145 inhibits tumor growth and metastasis by targeting metadherin in high-grade serous ovarian carcinoma. Oncotarget 2014; 5: 10816-10829.

[8] Wang Y, Yan S, Liu X, Zhang W, Li Y, Dong R, Zhang Q, Yang Q, Yuan C, Shen K and Kong B. miR-1236-3p represses the cell migration and invasion abilities by targeting ZEB1 in high-grade serous ovarian carcinoma. Oncol Rep 2014; 31: 1905-1910.

[9] Zhu X, Shen H, Yin X, Long L, Xie C, Liu Y, Hui L, Lin X, Fang Y, Cao Y, Xu Y, Li M, Xu W and Li Y. miR-186 regulation of Twist1 and ovarian cancer sensitivity to cisplatin. Oncogene 2016; 35: 323-332.

[10] Lu L, Katsaros D, Risch HA, Canuto EM, Biglia N and Yu H. MicroRNA let-7a modifies the effect of self-renewal gene HIWI on patient survival of epithelial ovarian cancer. Mol Carcinog 2016; 55: 357-365.

[11] Ma MZ, Chu BF, Zhang Y, Weng MZ, Qin YY, Gong W and Quan ZW. Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. Cell Death Dis 2015; 6: e1583.

[12] Chang L, Wang G, Jia T, Zhang L, Li Y, Han Y, Zhang K, Lin G, Zhang R, Li J and Wang L. Armored long non-coding RNA MEG3 targeting EGFR based on recombinant MS2 bacteriophage virus-like particles against hepatocellular carcinoma. Oncotarget 2016; 7: 23988-24004.

[13] Alaiyan B, Ilyayev N, Stojadinovic A, Izadjoo M, Roistacher M, Pavlov V, Tzivin V, Halle D, Pan H, Trink B, Gure AO and Nissan A. Differential expression of colon cancer associated transcript1 (CCAT1) along the colonic adenoma-carcinoma sequence. BMC Cancer 2013; 13: 196.

[14] Zhang Y, Zou Y, Wang W, Zuo Q, Jiang Z, Sun M, De W and Sun L. Down-regulated long non-coding RNA MEG3 and its effect on promoting apoptosis and suppressing migration of trophoblast cells. J Cell Biochem 2015; 116: 542-550.

[15] Wan G, Mathur R, Hu X, Liu Y, Zhang X, Peng G and Lu X. Long non-coding RNA ANRIL (CDKN2B-AS) is induced by the ATM-E2F1 signaling pathway. Cell Signal 2013; 25: 1086-1095.

[16] Huang MD, Chen WM, Qi FZ, Xia R, Sun M, Xu TP, Yin L, Zhang EB, De W and Shu YQ. Long non-coding RNA ANRIL is upregulated in hepatocellular carcinoma and regulates cell apoptosis by epigenetic silencing of KLF2. J Hematol Oncol 2015; 8: 50.

[17] Zhang EB, Kong R, Yin DD, You LH, Sun M, Han L, Xu TP, Xia R, Yang JS, De W and Chen J. Long noncoding RNA ANRIL indicates a poor prognosis of gastric cancer and promotes tumor

growth by epigenetically silencing of miR-99a/miR-449a. Oncotarget 2014; 5: 2276-2292.
[18] Wan X, Ding X, Chen S, Song H, Jiang H, Fang Y, Li P and Guo J. The functional sites of miRNAs and lncRNAs in gastric carcinogenesis. Tumour Biol 2015; 36: 521-532.
[19] Cao X, Zhuang S, Hu Y, Xi L, Deng L, Sheng H and Shen W. Associations between polymorphisms of long non-coding RNA MEG3 and risk of colorectal cancer in Chinese. Oncotarget 2016; 7: 19054-19059.
[20] Zhuo H, Tang J, Lin Z, Jiang R, Zhang X, Ji J, Wang P and Sun B. The aberrant expression of MEG3 regulated by UHRF1 predicts the prognosis of hepatocellular carcinoma. Mol Carcinog 2016; 55: 209-219.
[21] Peng W, Si S, Zhang Q, Li C, Zhao F, Wang F, Yu J and Ma R. Long non-coding RNA MEG3 functions as a competing endogenous RNA to regulate gastric cancer progression. J Exp Clin Cancer Res 2015; 34: 79.
[22] He A, Liu Y, Chen Z, Li J, Chen M, Liu L, Liao X, Lv Z, Zhan Y, Zhuang C, Lin J, Huang W and Mei H. Over-expression of long noncoding RNA BANCR inhibits malignant phenotypes of human bladder cancer. J Exp Clin Cancer Res 2016; 35: 125.
[23] Jiang W, Zhang D, Xu B, Wu Z, Liu S, Zhang L, Tian Y, Han X and Tian D. Long non-coding RNA BANCR promotes proliferation and migration of lung carcinoma via MAPK pathways. Biomed Pharmacother 2015; 69: 90-95.
[24] Tian ZZ, Guo XJ, Zhao YM and Fang Y. Decreased expression of long non-coding RNA MEG3 acts as a potential predictor biomarker in progression and poor prognosis of osteosarcoma. Int J Clin Exp Pathol 2015; 8: 15138-15142.
[25] Marques Howarth M, Simpson D, Ngok SP, Nieves B, Chen R, Siprashvili Z, Vaka D, Breese MR, Crompton BD, Alexe G, Hawkins DS, Jacobson D, Brunner AL, West R, Mora J, Stegmaier K, Khavari P, Sweet-Cordero EA. Long noncoding RNA EWSAT1-mediated gene repression facilitates Ewing sarcoma oncogenesis. J Clin Invest 2014; 124: 5275-90.
[26] Song P, Yin SC. Long non-coding RNA EWSAT1 promotes human nasopharyngeal carcinoma cell growth in vitro by targeting miR-326/-330-5p. Aging (Albany NY) 2016; 8: 2948-2960.
[27] Sun L, Yang C, Xu J, Feng Y, Wang L, Cui T. Long noncoding RNA EWSAT1 promotes osteosarcoma cell growth and metastasis through suppression of MEG3 expression. DNA Cell Biol 2016; 35: 812-818.