Clinical Significance of Galectin-7 in Epithelial Ovarian Cancer

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Abstract. *Background: Galectin-7 (GAL-7) has been highlighted as an important marker in many types of cancers by either inhibiting or promoting tumor growth. In this novel study, we assessed the association of GAL-7 with clinicopathological variables and survival outcomes in epithelial ovarian cancer (EOC) and investigated the role of GAL-7 in proliferation of ovarian cancer cell lines. Materials and Methods: The expression of GAL-7 was determined in 63 formalin-fixed, paraffin-embedded EOC tissues using an immunohistochemical method and we compared various associated clinicopathological factors. To evaluate the role of GAL-7 in cell proliferation, we performed proliferation assays with GAL-7 siRNA using ovarian cancer cell lines, including A2780-PAR cells. Results: Immunohistochemical analysis revealed that GAL-7 expression was primarily detected in nuclei and occasionally in the nucleus and cytoplasm. High GAL-7 expression was associated with greater age (p=0.016), high mortality (p=0.025), and poor overall survival outcome (p=0.029). In addition, the residual tumor volume was larger in the high-expression group compared to the low-expression group, although the difference was not statistically significant (p=0.059). Down-regulation of GAL-7 using siRNA resulted in the inhibition of cell proliferation of A2780-PAR cells. Conclusion: We observed that high GAL-7 might be associated with poor survival outcome in patients with EOC, and may be functionally involved in cell proliferation.*

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Epithelial ovarian cancer (EOC) remains one of the most common gynecological malignancies that results in death due to its typically advanced stage at the time of diagnosis (1, 2). Although many patients initially respond to the combinations of cytoreductive surgery and platinum/taxane chemotherapy, most patients experience subsequent recurrence (3). Therefore, investigation into the molecules that may be effective in improving survival and enhancing the response rate to chemotherapy is needed.

Galectins are members of the carbohydrate-binding lectin family characterized by their affinity for β-galactoside (4). A total of 15 different galectins have been identified and numbered in order of discovery (galectin-1 to galectin-15) (5). Galectins have been implicated in a wide range of cellular functions including embryonic development, wound healing, apoptosis, intercellular adhesion, cell migration, immune response, and malignant proliferation (5, 6). Galectin-7 (GAL-7), a member of the galectin family initially identified in human epidermis, is a 15-kDa protein with a single carbohydrate recognition domain (7, 8). The expression of GAL-7 is markedly altered in tumor cells compared to their normal counterparts (9, 10). GAL-7 has been shown to play an important role in cancer progression, dissemination, and invasion (9-12). Recently, GAL-7 was reported to be a key element in aggressive metastasis following its overexpression in breast carcinomas and represents an interesting molecule as a therapeutic target (13).

For ovarian cancer, several studies have shown that galectin expression was linked to poor prognosis (14-16), sensitivity to paclitaxel-based chemotherapy (17), tumor cell proliferation (16, 18), and cell invasion (16). However, the clinical significance of GAL-7 in patients with EOC is still unknown. Therefore, in this study, we investigated the prognostic significance of GAL-7 in patients with EOC and its functional role in cell proliferation in an ovarian cancer cell line.

Materials and Methods

Tumor samples. In this study, a total of 68 paraffin-embedded tissues were used. These included 63 specimens of EOC. As controls, we also obtained normal ovarian tissues (n=5) from patients who underwent hysterectomies for benign disease. All operations were performed at the Department of Obstetrics and Gynecology at Samsung Medical Center in Seoul, Korea between 1997 and 2005. All of the patients were treated with maximal debulking surgery, which was followed by administration of intravenous paclitaxel (175 mg/m^2) or docetaxel (75 mg/m^2) plus carboplatin [area under the curve (AUC) of 5] combination chemotherapy every three weeks for 6-8 cycles. We divided patients into two groups according to their sensitivity to the first-line platinum-based combination chemotherapy: i) platinum-resistant, as defined by a platinum-free interval of less six months, and ii) platinum-sensitive, as defined by platinum-free interval greater than or equal six months. Surgical staging was established according to the International Federation of Gynecology and Obstetrics (FIGO) system (19). Optimal cytoreduction was defined as no grossly visible tumor at the completion of the surgical procedure. Sub-optimal or non-complete cytoreduction was defined as residual tumor measuring >0 cm at the completion of the surgical procedure. All samples were collected according to the Institutional Review Board of the Samsung Medical Center (IRB number: 2009-09-002-002).

Immunohistochemistry. Immunohistochemical studies were carried out on formalin-fixed, paraffin-embedded 4-μm-thick tissue sections. The primary antibodies used were rabbit polyclonal antibodies against GAL-7 (a gift from Dr. Sabine Andre, Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Munich, Germany). Tissue sections were de-paraffinized three times in xylene for a total of 15 min and subsequently rehydrated. Antigen retrieval was carried out at 97°C, with PTLink (DAKO, Glostrup, Denmark) for 20 min in citrate buffer (pH 6.0). After blocking endogenous peroxidase activity with 3% hydrogen peroxidase for 10 min, the primary antibody incubation for GAL-7 was carried out for 120 min at room temperature, with a dilution of 3 μl/ml. The antigen–antibody reaction was detected using the DAKO REAL TM EnvisionTM Detection system, Peroxidase/DAB K5007 (DAKO). Counterstaining was performed with Mayer's hematoxylin. Staining for GAL-7 was considered positive when tumor cells exhibited nuclear and/or cytoplasmic reactivity. Negative controls [substituting Tris-buffered saline (TBS) for primary antibody] were run simultaneously. Two pathologists (Dr.C.O.Sung and Dr.I.G.Do) without previous knowledge of the clinical outcomes assessed each slide. The intensity of staining was graded on a semi quantitative scale from 0 to 3, where 0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining. The percentage of positive cells was stratified from 0 to 3, where 0%=0, 1-20%=1, 21-50%=2, 51-100%=3 (20). The total score was calculated by multiplying the intensity score and the stratified score for the percentage of positive cells and this ranged from 0 to 9. Scores from 0 to 4 were considered low and scores from 5 to 9 were considered high.

Cell lines. Human EOC cells (HeyA8, SKOV3ip1 and A2780-PAR) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The taxol-resistant EOC cells (HeyA8-MDR, SKOV3-TR) and the cisplatin-resistant EOC cells (A2780-CP20) were a gift from Dr. Anil K. Sood, Department of Cancer Biology, University of Texas M.D. Anderson Cancer Center, TX, USA.

Human EOC cell lines were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 0.1% gentamicin sulfate (Gemini Bioproducts, Calabasas, CA, USA) in 5% CO_2 at 37°C.

Transfection of GAL-7 siRNA. GAL-7 siRNA and negative control siRNA were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A2780-PAR cells were seeded at 3×10^3 cells per well in a 96-well microplate in culture media with 10% FBS. siRNA was transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Cells were then incubated at 37°C for 72 h.

Western blot analysis. Cells were lysed in PRO-PRE Protein Extraction Solution (Intron Biotechnology, Seongnam, Korea). Protein lysates were separated on 15% acrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Hybond-enhanced chemiluminescence (ECL) nitrocellulose filter paper (Amersham, Little Chalfont, Buckinghamshire, UK). Membranes were blocked with 5% skimmed milk in Tris-buffered saline, containing 0.1% Tween-20 for 1 h at room temperature. Protein bands were probed with an antibody against GAL-7 (Abcam, Cambridge, UK), and α-tubulin (Epitomics, Burlingame, CA, USA), and then labeled with horseradish peroxidase-conjugated anti-rabbit antibody (Amersham, Piscataway, NJ, USA). Bands were visualized using an ECL kit (Amersham), according to the manufacturer's protocol.

Isolation of total RNA, cDNA synthesis, and RT-PCR. Total RNA from each cell line was extracted using an Ambion mirVana miRNA isolation Kit (Ambion, Austin, TX, USA). First-stranded cDNA was synthesized by reverse transcriptase using the High Capacity cDNA RT Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The cDNA was amplified by PCR using the following primer sequences for GAL-7: 5'-ACCAACCCGG TCCCAG-3' (forward) and 5'-GCGGGCTAACG CTTTATTTGC-3' (reverse) (13). An endogenous control cDNA was formed using β-actin primers: 5'-GATGCAGAAGGAGATCACTG-3' (forward) and 5'-AGTCATAGTCCGCCTAGAAG-3' (reverse). PCR was carried out by initial denaturation at 95°C for 5 min, followed by either 35 cycles or 28 cycles of denaturation (95°C, 40 s), annealing (68°C for GAL-7, 57°C for β-actin, 40 s), and extension (72°C, 1 min) for GAL-7 and β-actin, respectively. This was followed by a final extension step at 72°C for 10 min. Amplification products were electrophoresed on 1% agarose gels and visualized by ethidium bromide staining under ultraviolet transillumination.

Proliferation assay. For the proliferation assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) solution (Amresco, Solon, OH, USA) was subsequently added to each well. After an additional 4 h of incubation, the medium was discarded, 100 μl of acidic isopropanol (0.1 N HCl in absolute isopropanol) was added, and the plate was shaken gently. Absorbance was measured on an enzyme-linked immunosorbent assay (ELISA) reader at a test wavelength of 540 nm.

Statistical analysis. Fisher's exact probability test or the chi-square test was used to assess for statistical significance between GAL-7 expression and clinicopathological parameters. The Fisher's exact test was used if the expected frequency was less than five. Kaplan-Meier curves were plotted to assess the effects of GAL-7 expression on survival. These survival curves were compared using the log-rank test. Variables shown

Table I. *Galectin-7 (GAL-7) expression in epithelial ovarian cancer based on immunohistochemistry.*

Cell type	Intensity of GAL-7 staining, n (%)				Total
	0	1	2	3	
Normal	0	5 (100)	0	0	5
Serous	0	1 (2.0)	32 (62.7)	18 (35.3)	51
Endometrioid	0	0	7 (77.8)	2 (22.2)	10
Mucinous	0	0	3 (100)	0	3

Table II. *Clinicopathological findings according to galectin-7 (GAL-7) expression.*

	GAL-7 expression		*p*-Value
	Score 0-4 (n=18)	Score 5-9 (n=45)	
Age (years), median	45.0 (38-74)	54.0 (31-78)	0.016
Stage			0.360
I/II	6 (33.3%)	10 (22.2%)	
III/IV	12 (66.7%)	35 (77.8%)	
Histology			0.299
Serous	13 (72.2%)	38 (84.4%)	
Non-serous	5 (27.8%)	7 (15.6%)	
Grade			1.0
I/II	4 (22.2%)	9 (20.0%)	
III	13 (72.2%)	34 (75.6%)	
Unknown	1 (5.5%)	2 (0.04%)	
Residual tumor			0.059
Microscopic	11 (61.1%)	13 (28.9%)	
0-1 cm	3 (16.7%)	11 (24.4%)	
≥1 cm	4 (22.2%)	21 (46.7%)	
Chemosensitivity			1.0
Sensitive	16 (88.9%)	35 (85.4%)	
Resistant	2 (11.1%)	6 (14.6%)	
Recurrence			0.451
No	8 (44.4%)	14 (34.1%)	
Yes	10 (55.6)	27 (65.9%)	
Death			0.025
No	14 (77.8%)	21 (46.7%)	
Yes	4 (22.2%)	24 (53.3%)	
Median PFS time, months (range)	34.9 (0.7-102.4)	18.1 (0.2-117.7)	0.280
Median OS time, months (range)	72.8 (11.2-106.7)	56.5 (0.2-121.8)	0.029

OS: Overall survival; PFS: progression-free survival.

to be significant or borderline-significant (*p*<0.25) in the univariate analysis were selected for the Cox model. *p*-Values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS (version 10.0; SPSS, Inc, Chicago, IL, USA).

Results

Immunohistochemical staining of GAL-7 in ovarian cancer. Immunohistochemical staining for GAL-7 was performed in both normal ovarian tissues (n=5) and EOC samples (n=63). Immunohistochemical staining of normal ovarian tissues showed that the cytoplasm and nuclei of epithelial cells were weakly-positive for GAL-7. The expression of GAL-7 in EOC tissues was primarily detected in cell nuclei and occasionally in the nucleus and cytoplasm (Figure 1). Moreover, GAL-7 was highly expressed in EOC tissues relative to normal ovarian samples.

Comparison of clinicopathological findings with GAL-7 expression. We divided all of the cancer patients into two groups according to the total immunohistochemical score using a cut-off of 5 (<5, low score *vs*. ≥5, high score). The FIGO stage, grade, histological type, and chemosensitivity were not significantly different between the two groups. The median age, however, was approximately nine years older in the high-expression group, which was statistically significant. In addition, the residual tumor volume was larger in the high expression group compared to the low expression group, although the difference was not statistically significant (*p*=0.059) (Table II).

The overall survival (OS) for all patients was analyzed based upon GAL-7 expression. The median OS was 72 months for patients with low GAL-7 expression and only 56 months for patients with high GAL-7 expression. Patients with high GAL-7 expression had significantly shorter OS than patients with low GAL-7 expression (*p*=0.029) (Figure 2). One patient in the low expression group had stage IIIC high-grade serous ovarian cancer with sub-optimal debulking at initial surgery followed by platinum-based chemotherapy. She eventually experienced recurrence and died within 90 months. Univariate analysis revealed that greater age, advanced stage of cancer, platinum resistance, sub-optimal cytoreduction, and high GAL-7 expression predicted a poorer OS. In multivariate analysis, advanced stage of cancer, platinum resistance and high GAL-7 expression were consistently independent prognostic factors for poor OS in these patients (Table III).

In vitro down-regulation of GAL-7 using its siRNA. We used western blot analysis to assess the expression of GAL-7 in several human ovarian cancer cell lines, including HeyA8, HeyA8-MDR, SKOV3ip1, SKOV3TR, A2780-PAR, and A2780-CP20 cells. The expression in the examined cells ranged from weak to strong (Figure 3A). To evaluate the proliferative capability of ovarian cancer cells according to GAL-7 expression, we performed proliferation assays using A2780-PAR and A2780-CP20 cells. We found that A2780-PAR cells, which had high GAL-7 expression, exhibited high proliferation compared with A2780-CP20 cells (Figure 3B). Among cell lines with relatively high expression of GAL-7, we decided to select A2780-PAR cells to perform an *in vitro*
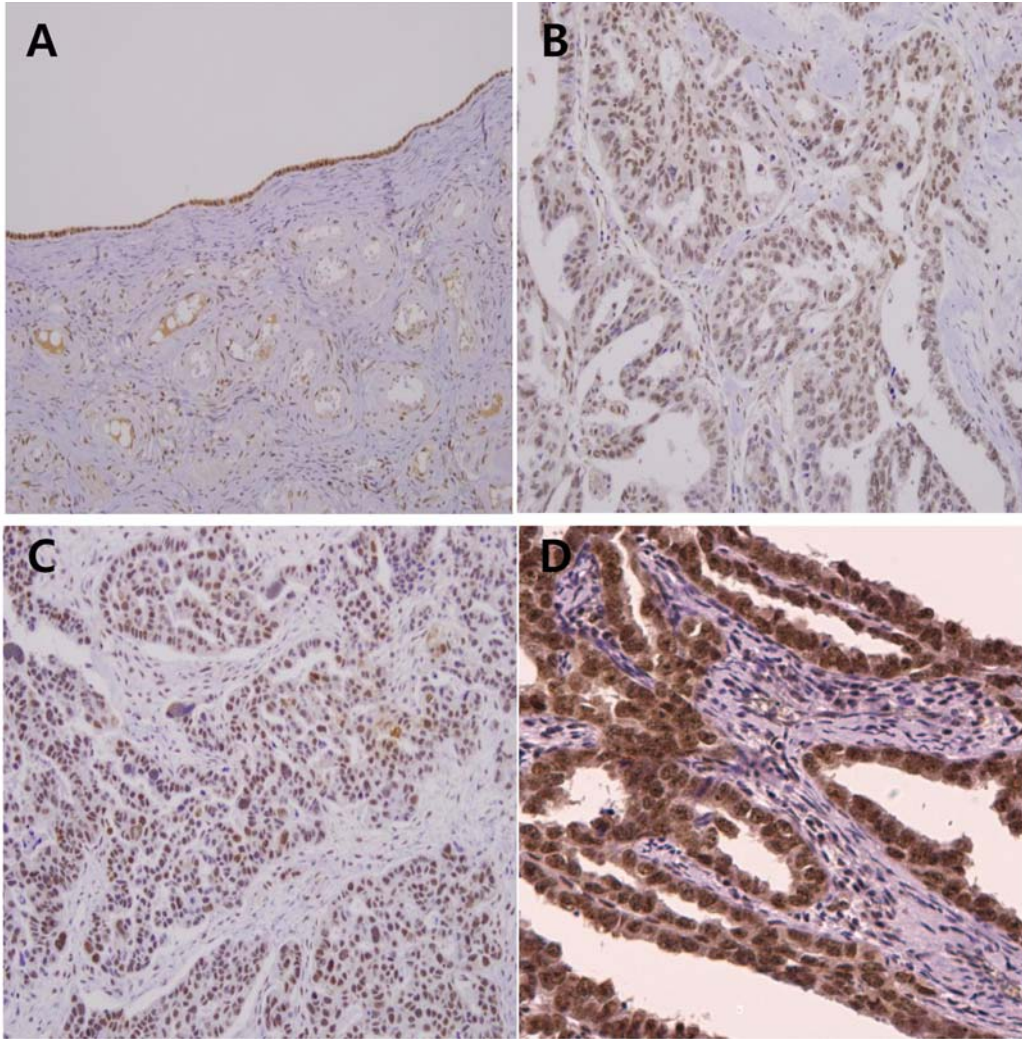
Figure 1. *Immunohistochemical evaluation of galectin-7 (Gal-7) expression in ovarian tissues. Representative examples of immunohistochemical results: Normal ovarian tissue showing weak GAL-7 expression (A); serous epithelial ovarian cancer (EOC) showing weak GAL-7 expression (B); endometrioid EOC showing moderate GAL-7 expression (C); serous EOC showing strong GAL-7 expression(D); Original magnification ×200.*

experiment using GAL-7 siRNA. We found that GAL-7 siRNA efficiently induced down-regulation of GAL-7 expression in a dose-dependent manner in A2780-PAR cells (Figure 3C).

To evaluate the role of GAL-7 in cell proliferation, we performed MTT assays using A2780-PAR cells transfected with GAL-7 siRNA. We found that GAL-7 siRNA had a growth-inhibiting effect at two or three days after treatment of A2780-PAR cells (Figure 3D).

Discussion

In this study, we explored the impact of GAL-7 in EOC using both patient samples and cell lines. We demonstrated that GAL-7 expression in EOC was up-regulated compared to normal ovarian tissues and high GAL-7 expression was associated with greater age, high mortality, and poor overall survival outcome. In addition, the residual tumor volume was larger in the high-expression group compared to the low expression group. *In vitro* data indicated that down-regulation of GAL-7 expression using its siRNA inhibited tumor cell proliferation. To the best of our knowledge, this is the first study regarding the role of GAL-7 in ovarian cancer.

Since GAL-7 has been primarily found to be a regulator of differentiation and apoptosis, one would expect that GAL-7 expression should favor the elimination of tumor cells during cancer progression (21-23). However, high expression of GAL-7 has been reported in chemically-induced rat mammary tumors (24), human malignant thyroid tissues (25), type IV hypopharyngeal squamous cell carcinoma (10), and aggressive lymphoma cells (26). The results of the
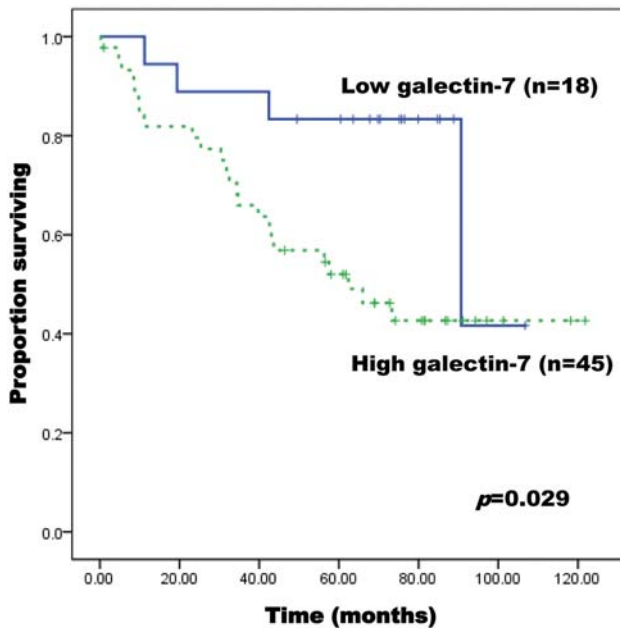
Figure 2. *Overall survival curves of patients according to the staining intensity of galectin-7.*

present study concerning GAL-7 expression in EOC was well in line with previous studies.

Several studies found that up-regulation of GAL-7 in lymphoma cells played a critical role in progression, dissemination, and invasion (11, 26, 27). Moisan *et al*. reported that up-regulation of Gal-7 in murine lymphoma cells was associated with progression towards an aggressive phenotype (27). Demers *et al*. demonstrated that mice injected with lymphoma cells, expressing Gal-7, developed large metastatic tumors in the liver and kidneys with massive infiltration of tumor cells in the parenchyma (26). Recent work in breast cancer supported the idea that GAL-7 may promote tumorigenesis (13). High expression of GAL-7 in breast cancer cells increased their ability to metastasize to the lungs and bones. Moreover, GAL-7 expression was associated with high-grade tumor, human epidermal growth factor-2 (HER2) overexpression, and lymph node axillary metastasis. Our results are consistent with the above studies. The present study demonstrates the correlation between GAL-7 expression levels and various clinicopathological factors. Patients with high expression of GAL-7 had tumor stage IIIC or IV in 71.1% (32/45) of cases compared to those with low expression at 55.6% (10/18) (*p*=0.237). Moreover, the levels of GAL-7 expression were positively correlated with residual tumor volume and poor OS of patients with EOC (*p*=0.059 and *p*=0.029, respectively).

Although most EOCs respond to chemotherapy initially, patients may experience recurrence with newly acquired drug resistance. In this study, we found no association between GAL-7 expression and chemosensitivity in EOC. It was recently reported that GAL-7 plays an important role acting downstream of p53 in urothelial cancer cells and may influence chemosensitivity to *cis*-diamminedichloroplatinum (CDDP) (28). They demonstrated that GAL-7 transfection into bladder cancer cells sensitized cancer cells with mutant p53 to CDDP *via* the promotion of intracellular reactive oxygen species (ROS) generation. Moreover, they suggested that GAL-7 did not seem to cause apoptosis nor change cellular proliferation but promoted cell susceptibility to genotoxic stress caused by CDDP exposure. Given that little is known about the function of GAL-7 in relation to chemosensitivity, further study is needed to firmly draw such a conclusion.

A few studies have examined the mechanisms underlying the tumorigenic effects of GAL-7. They suggested that one mechanism of the pro-tumor role of GAL-7 may be *via* an increase in production of matrix metalloproteinases-9 (MMP-9), which plays an important role in cancer progression and metastasis. GAL-7 up-regulated MMP-9 expression and cell invasion of lymphoma cells (26) and cervical adenocarcinoma cells (29). In laryngeal and hypopharyngeal cancer, there was a positive correlation of MMP-9 with GAL-7 (30). Another mechanism may relate to cancer cell migration. The galectin-mediated modulation of cancer cell migration may be associated with integrins in cell-to-cell and cell-to-extracellular matrix interactions. In Hela cells transfected with GAL-7, a four-fold increase in $\alpha 1$ integrin mRNA was observed (22). Cao *et al*. demonstrated a number of potential roles for GAL-7 as a mediator of corneal epithelial cell migration (31).

A limitation of our study was the relatively small population of patients that we evaluated. Furthermore, we did not identify the molecular mechanisms underlying the effects of GAL-7 regarding tumor cell proliferation. Further research is required to determine the mechanisms underlying the tumorigenic effects of GAL-7.

In conclusion, this study suggests that high GAL-7 expression may be related to poor prognosis in EOC and that GAL-7 may be functionally involved in cell proliferation. Future studies should address the utility of GAL-7 as a useful therapeutic target in the treatment of EOC.

Conflicts of Interest

The Authors declare no conflicts of interest.
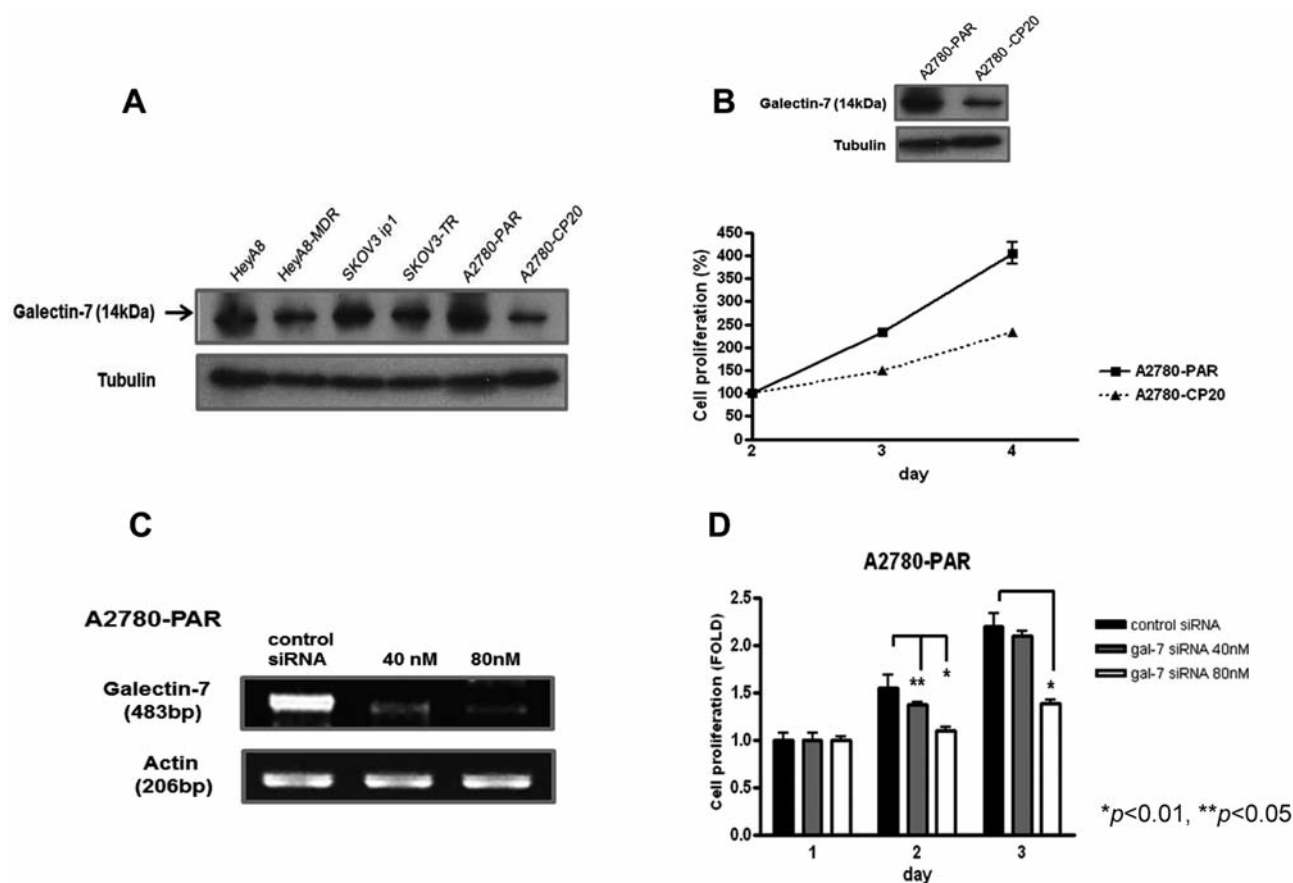
Acknowledgements

Figure 3. *Expression of galectin-7 (GAL-7) in various ovarian cancer cell lines and in vitro cell proliferation assays using siRNA. A: Basal expression of GAL-7 in ovarian cancer cell lines. B: A2780-PAR cells, which had high GAL-7 expression, exhibited high proliferation compared with A2780-CP20 cells. C: The expression of GAL-7 was reduced by transfecting A2780-PAR cells with GAL-7 siRNA. D: Proliferation assay using GAL-7 siRNA showed that cell proliferation was significantly reduced in the transfected A2780-PAR cell line. *p<0.01; **p<0.05.*

Table III. *Univariate and multivariate analyses of overall survival according to individual parameters.*

Parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age (<55y *vs.* ≥55y)	1.05 (1.01-1.08)	0.006	0.97 (0.93-1.02)	0.244
Stage (I/II *vs.* III/IV)	13.26 (1.80-97.7)	0.001	15.2 (1.66-139.9)	0.016
Histology (serous *vs.* non-serous)	0.45 (0.14-1.49)	0.180	1.65 (0.45-6.07)	0.450
Residual tumor (microscopic *vs.* >0 cm)	2.90 (1.17-7.18)	0.016	0.70 (0.2-2.43)	0.572
Chemosensitivity (sensitive *vs.* resistant)	12.1 (4.78-30.8)	<0.001	10.9 (2.64-45.5)	0.001
GAL-7 expression (0-4 *vs.* 5-9)	3.07 (1.06-8.87)	0.029	4.56 (1.24-16.7)	0.022

HR, Hazard ratio; CI, confidence interval.

References

1   Landen CN, Jr., Birrer MJ and Sood AK: Early events in the pathogenesis of epithelial ovarian cancer. J Clin Oncol *26*: 995-1005, 2008.

2   Ozols RF, Bookman MA, Connolly DC, Daly MB, Godwin AK, Schilder RJ, Xu X and Hamilton TC: Focus on epithelial ovarian cancer. Cancer Cell *5*: 19-24, 2004.

3   du Bois A, Luck HJ, Meier W, Adams HP, Mobus V, Costa S, Bauknecht T, Richter B, Warm M, Schroder W, Olbricht S, Nitz

U, Jackisch C, Emons G, Wagner U, Kuhn W and Pfisterer J: A randomized clinical trial of cisplatin/paclitaxel *versus* carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst *95*: 1320-1329, 2003.

4 Barondes SH, Cooper DN, Gitt MA and Leffler H: Galectins. Structure and function of a large family of animal lectins. J Biol Chem *269*: 20807-20810, 1994.

5 Liu FT and Rabinovich GA: Galectins as modulators of tumour progression. Nat Rev Cancer *5*: 29-41, 2005.

6 Danguy A, Camby I and Kiss R: Galectins and cancer. Biochim Biophys Acta *1572*: 285-293, 2002.

7 Madsen P, Rasmussen HH, Flint T, Gromov P, Kruse TA, Honore B, Vorum H and Celis JE: Cloning, expression, and chromosome mapping of human galectin-7. J Biol Chem *270*: 5823-5829, 1995.

8 Magnaldo T, Bernerd F and Darmon M: Galectin-7, a human 14-kDa S-lectin, specifically expressed in keratinocytes and sensitive to retinoic acid. Dev Biol *168*: 259-271, 1995.

9 Chen J, He QY, Yuen AP and Chiu JF: Proteomics of buccal squamous cell carcinoma: The involvement of multiple pathways in tumorigenesis. Proteomics *4*: 2465-2475, 2004.

10 Saussez S, Cucu DR, Decaestecker C, Chevalier D, Kaltner H, Andre S, Wacreniez A, Toubeau G, Camby I, Gabius HJ and Kiss R: Galectin-7 (p53-induced gene 1): a new prognostic predictor of recurrence and survival in stage IV hypopharyngeal cancer. Ann Surg Oncol *13*: 999-1009, 2006.

11 Demers M, Biron-Pain K, Hebert J, Lamarre A, Magnaldo T and St-Pierre Y: Galectin-7 in lymphoma: Elevated expression in human lymphoid malignancies and decreased lymphoma dissemination by antisense strategies in experimental model. Cancer Res *67*: 2824-2829, 2007.

12 Saussez S, Decaestecker C, Lorfevre F, Chevalier D, Mortuaire G, Kaltner H, Andre S, Toubeau G, Gabius HJ and Leroy X: Increased expression and altered intracellular distribution of adhesion/growth-regulatory lectins galectins-1 and -7 during tumour progression in hypopharyngeal and laryngeal squamous cell carcinomas. Histopathology *52*: 483-493, 2008.

13 Demers M, Rose AA, Grosset AA, Biron-Pain K, Gaboury L, Siegel PM and St-Pierre Y: Overexpression of galectin-7, a myoepithelial cell marker, enhances spontaneous metastasis of breast cancer cells. Am J Pathol *176*: 3023-3031, 2010.

14 Brustmann H: Epidermal growth factor receptor expression in serous ovarian carcinoma: an immunohistochemical study with galectin-3 and cyclin D1 and outcome. Int J Gynecol Pathol *27*: 380-389, 2008.

15 Kim MK, Sung CO, Do IG, Jeon HK, Song TJ, Park HS, Lee YY, Kim BG, Lee JW and Bae DS: Overexpression of Galectin-3 and its clinical significance in ovarian carcinoma. Int J Clin Oncol *16*: 352-358, 2011.

16 Kim HJ, Jeon HK, Cho YJ, Park YA, Choi JJ, Do IG, Song SY, Lee YY, Choi CH, Kim TJ, Bae DS, Lee JW and Kim BG: High galectin-1 expression correlates with poor prognosis and is involved in epithelial ovarian cancer proliferation and invasion. Eur J Cancer *48*: 1914-1921, 2012.

17 Oishi T, Itamochi H, Kigawa J, Kanamori Y, Shimada M, Takahashi M, Shimogai R, Kawaguchi W, Sato S and Terakawa N: Galectin-3 may contribute to cisplatin resistance in clear cell carcinoma of the ovary. Int J Gynecol Cancer *17*: 1040-1046, 2007.

18 van den Brule F, Califice S, Garnier F, Fernandez PL, Berchuck A and Castronovo V: Galectin-1 accumulation in the ovary

carcinoma peritumoral stroma is induced by ovary carcinoma cells and affects both cancer cell proliferation and adhesion to laminin-1 and fibronectin. Lab Invest *83*: 377-386, 2003.

19 Changes in definitions of clinical staging for carcinoma of the cervix and ovary: International Federation of Gynecology and Obstetrics. Am J Obstet Gynecol *156*: 263-264, 1987.

20 Chui X, Egami H, Yamashita J, Kurizaki T, Ohmachi H, Yamamoto S and Ogawa M: Immunohistochemical expression of the c-KIT proto-oncogene product in human malignant and non-malignant breast tissues. Br J Cancer *73*: 1233-1236, 1996.

21 Ostergaard M, Rasmussen HH, Nielsen HV, Vorum H, Orntoft TF, Wolf H and Celis JE: Proteome profiling of bladder squamous cell carcinomas: Identification of markers that define their degree of differentiation. Cancer Res *57*: 4111-4117, 1997.

22 Kuwabara I, Kuwabara Y, Yang RY, Schuler M, Green DR, Zuraw BL, Hsu DK and Liu FT: Galectin-7 (PIG1) exhibits pro-apoptotic function through JNK activation and mitochondrial cytochrome c release. J Biol Chem *277*: 3487-3497, 2002.

23 Kopitz J, Andre S, von Reitzenstein C, Versluis K, Kaltner H, Pieters RJ, Wasano K, Kuwabara I, Liu FT, Cantz M, Heck AJ and Gabius HJ: Homodimeric galectin-7 (p53-induced gene 1) is a negative growth regulator for human neuroblastoma cells. Oncogene *22*: 6277-6288, 2003.

24 Lu J, Pei H, Kaeck M and Thompson HJ: Gene expression changes associated with chemically induced rat mammary carcinogenesis. Mol Carcinog *20*: 204-215, 1997.

25 Rorive S, Eddafali B, Fernandez S, Decaestecker C, Andre S, Kaltner H, Kuwabara I, Liu FT, Gabius HJ, Kiss R and Salmon I: Changes in galectin-7 and cytokeratin-19 expression during the progression of malignancy in thyroid tumors: Diagnostic and biological implications. Mod Pathol *15*: 1294-1301, 2002.

26 Demers M, Magnaldo T and St-Pierre Y: A novel function for galectin-7: Promoting tumorigenesis by up-regulating *MMP-9* gene expression. Cancer Res *65*: 5205-5210, 2005.

27 Moisan S, Demers M, Mercier J, Magnaldo T, Potworowski EF and St-Pierre Y: Up-regulation of galectin-7 in murine lymphoma cells is associated with progression toward an aggressive phenotype. Leukemia *17*: 751-759, 2003.

28 Matsui Y, Ueda S, Watanabe J, Kuwabara I, Ogawa O and Nishiyama H: Sensitizing effect of galectin-7 in urothelial cancer to cisplatin through the accumulation of intracellular reactive oxygen species. Cancer Res *67*: 1212-1220, 2007.

29 Park JE, Chang WY and Cho M: Induction of matrix metalloproteinase-9 by galectin-7 through p38 MAPK signaling in HeLa human cervical epithelial adenocarcinoma cells. Oncol Rep *22*: 1373-1379, 2009.

30 Saussez S, Cludts S, Capouillez A, Mortuaire G, Smetana K Jr., Kaltner H, Andre S, Leroy X, Gabius HJ and Decaestecker C: Identification of matrix metalloproteinase-9 as an independent prognostic marker in laryngeal and hypopharyngeal cancer with opposite correlations to adhesion/growth-regulatory galectins-1 and -7. Int J Oncol *34*: 433-439, 2009.

31 Cao Z, Said N, Wu HK, Kuwabara I, Liu FT and Panjwani N: Galectin-7 as a potential mediator of corneal epithelial cell migration. Arch Ophthalmol *121*: 82-86, 2003.