

Research Article

# Predictive role of galectin-1 and integrin $\alpha 5\beta 1$ in cisplatin-based neoadjuvant chemotherapy of bulky squamous cervical cancer

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Although galectin-1 and integrin  $\alpha 5\beta 1$  confer chemoresistance to certain types of cancer, whether their expression predicts the response to cisplatin-based neoadjuvant chemotherapy (NACT) in squamous cervical cancer remains unclear. Paired tumor samples (pre- and post-chemotherapy) were obtained from 35 bulky squamous cervical cancer patients treated with cisplatin-based NACT and radical hysterectomy at our hospital between January 2007 and August 2014. The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in tumor cells and stromal cells was analyzed by immunohistochemistry. The correlation between galectin-1/integrin  $\alpha 5\beta 1$  and apoptosis-associated markers was investigated by using the The Cancer Genome Atlas (TCGA) RNA-sequencing data. Seventeen patients were identified as chemotherapy responders and 18 as non-responders. Galectin-1 and integrin  $\alpha 5\beta 1$ -positive immunostaining was more frequently observed in stromal cells than its in tumor cells. The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal and tumor cells was significantly down-regulated in postchemotherapy cervical cancer tissues. High levels of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal were associated with a negative chemotherapy response in squamous cervical cancer patients treated with cisplatin-based NACT. Additionally, the expression of galectin-1 and integrin  $\alpha 5$  correlated negatively with caspase 3/caspase 8 by using the TCGA RNA-sequencing data. Galectin-1 and integrin  $\alpha 5\beta 1$  expression in stromal may serve as a prediction of the responses to cisplatin-based NACT for patients with bulky squamous cervical cancer. Galectin-1 and integrin  $\alpha 5\beta 1$  may be implicated in the development of chemoresistance in cervical cancer via suppressing apoptosis.

## Introduction

Cervical cancer is the second most commonly diagnosed cancer and the third leading cause of cancer deaths amongst females in less developed countries, with an estimated 527600 new cases and 265700 deaths worldwide in 2012 [1]. Even in the United States, where the prevention of cervical cancer by Pap smear based screening and treatment programs have been largely successful, approximately 12900 new cases and 4100 deaths from cervical cancer were estimated for 2015 [2]. Specifically, the prognosis of locally advanced cervical cancer (tumor size  $\geq 4$  cm) was very poor after conventional treatments, with a 5-year overall survival rate of approximately 40% [3]. Recently, neoadjuvant chemotherapy (NACT) prior to surgery has been widely used for bulky or locally advanced cervical cancer. This new therapeutic strategy can shrink the tumor volume, control micrometastases and improve the safety and integrity of surgery

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Received: 19 June 2017  
Revised: 22 August 2017  
Accepted: 23 August 2017

Accepted Manuscript Online:  
25 August 2017  
Version of Record published:  
28 September 2017

[3]. Nevertheless, the prognosis of NACT-refractory patients would become worse due to the delay in curative treatment [4]. Currently, there is no method to reliably predict tumor responses to NACT prior to the therapy. Therefore, it is of great clinical value to identify and develop effective biomarkers that can predict the resistance to NACT and thus to assess the treatment options of individual patients.

In our previous study, we have identified 16 proteins, including galectin-1, which were down-regulated in squamous cervical cancer tissue after NACT relative to the level before chemotherapy by using Proteomic Profiling [5]. Galectin-1, belonging to the  $\beta$ -galactoside-binding lectin family, is well known to be involved in a wide range of physiologic processes such as cell transformation, cell proliferation, angiogenesis, cell adhesion and invasiveness, and immunosuppression [6]. Indeed, accumulating studies have explored its pivotal role in the carcinogenesis of cervix. Kohrenhagen et al. [7] reported that galectin-1 expression on stromal cells increased with the histopathologic grade of cervical tissues. High galectin-1 expression in peritumoral stroma was significantly correlated with the depth of invasion in the cervix and lymph node metastasis [8]. Galectin-1 inhibition by siRNA or galectin-1 antibody suppressed the growth of cervical cancer both *in vitro* and *in vivo* [8,9]. Furthermore, galectin-1 is an independent prognostic factor for local recurrence and survival in stage I–II cervical cancer patients undergoing definitive radiation therapy [10].

Recent evidence indicates that galectin-1 exerts a potent role in the development of chemoresistance. Suppression of galectin-1 can sensitize lung cancer cells to cisplatin [11,12], while increase the expression of galectin-1 in epithelial ovarian cells can enhance their resistance to cisplatin [13]. However, whether galectin-1 expression correlates with chemoresistance in squamous cervical cancer patients is currently unknown.

Integrins are heterodimeric transmembrane adhesion receptors, which contain two different chains,  $\alpha$  and  $\beta$  subunits [14]. Integrins mediate a wide variety of cell–cell and cell–matrix interactions that lead to cell migration, proliferation, differentiation, and survival. Integrin  $\alpha 5$  primarily combines with integrin  $\beta 1$  subunit to form  $\alpha 5\beta 1$  heterodimer [15]. Integrin  $\alpha 5\beta 1$  is one of the integrin family members and could play a role in cell survival and chemoresistance in certain types of cancer [16–18]. With respect to human cervical cancer, integrin  $\alpha 5\beta 1$  has been detected overexpressed in cervical cancer tissues, and high levels of integrin  $\alpha 5\beta 1$  were correlated with poor differentiation, lymph node metastasis, recurrence as well as a poor prognosis in cervical cancer [19]. However, none of the studies has evaluated the possible role of integrin  $\alpha 5\beta 1$  in chemotherapy responses amongst cervical cancer patients.

In the present study, we first examined the correlation between the expression of galectin-1 and integrin  $\alpha 5\beta 1$  and the responses to cisplatin-based NACT in patients with stage IB2 or IIA2 squamous cervical cancer, and then explored their potential mechanism(s) using the The Cancer Genome Atlas (TCGA) and GEO RNA-sequencing data.

## Materials and methods

### Ethics statement

The present study was approved by the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University and conducted according to the Helsinki Declaration. Written informed consents were granted from all the subjects at the time of enrollment.

### Patients and tissue specimens

Patients with IB2 or IIA2 (bulky, primary tumor >4 cm in diameter) squamous cervical cancer at the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, between January 2007 and August 2014, were recruited for a pilot study aimed to identify predictive biomarkers for responses to NACT. Patients were included if they met the following criteria: (i) Patients had confirmed diagnosis of squamous cervical cancer by pathology. (ii) The lesions in non-pregnant patients who had normal bone marrow, liver, and kidney functions were measured by physical examination, transvaginal ultrasound, and MRI. Only individuals with bulky stage IB2 or IIA2 cervical cancer (primary tumor >4 cm in diameter) were included. (iii) Patients who did not complete the planned cycles of NACT were excluded. (iv) Patients receiving chemotherapy, immunotherapy, hormonal therapy, or radiotherapy before the specimen collection were excluded. Finally, a total of 35 subjects were enrolled with a median age of 46 years (range: 25–63 years).

Paired tumor samples from each patient were obtained during cervical biopsy (prechemotherapy) or surgery (postchemotherapy). The biopsies from each patient were put into 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), embedded in paraffin, and cut into 4- $\mu$ m sections. Then, hematoxylin and eosin stained slides of the cervical tumor were reviewed for confirmation of pathological features, as well as to select suitable tissue blocks for immunohistochemical analysis.

## NACT and therapeutic effect evaluation

All eligible patients received one or two courses of cisplatin-based NACT, as previously described [20]: cisplatin, 60 mg/m<sup>2</sup> on day 1; 5-fluorouracil, 750 mg/m<sup>2</sup> on day 1; mitomycin, 8 mg/m<sup>2</sup> on day 1 for one or two courses, every 28 days. All these chemotherapeutics were administered via uterine artery injection. The chemotherapy response was evaluated by measuring the tumor's two dimensions (the longest diameter and its perpendicular diameter) with MRI or other radiographic means. The chemotherapy response was determined 2 weeks after completion of NACT, according to the WHO criteria [21]. Complete response (CR) was defined as the complete remission of the tumor. Partial response (PR) was defined as at least a 50% decrease in the tumor volume. Stable disease (SD) meant a steady state or a response less than 50%, and progressive disease (PD) was defined as an unequivocal increase in at least 25% of the tumor volume. The patients with CR or PR were defined as chemotherapy responders, while the patients with SD or PD were deemed as chemotherapy non-responders. All patients were treated with radical hysterectomy and bilateral pelvic lymphadenectomy 2–3 weeks after completion of the NACT regimen as described previously [4].

## Immunohistochemistry

Immunohistochemical staining for galectin-1 and integrin  $\alpha 5\beta 1$  was performed in all the cases. Biopsy was embedded in paraffin, cut into 4- $\mu$ m sections, and then mounted on to poly-L-lysine-coated slides. Briefly, after deparaffinization in xylenes and rehydration through graded ethanol solutions, sections were boiled in 10  $\mu$ mol/l citric buffer solution (pH 6.0) in a microwave oven for 10 min, followed by incubation with 3% hydrogen peroxide in methanol to suppress the endogenous peroxidase activity and overnight incubation with primary antibodies at 4°C. The following primary antibodies and corresponding dilutions were used: galectin-1 (Abcam, U.S.A.; 1:200) and integrin  $\alpha 5\beta 1$  (Millipore, U.S.A.; 1:200). Subsequently, the sections were incubated with prediluted biotinylated goat anti-mouse or goat anti-rabbit secondary antibody (Santa Cruz Technology, U.S.A.) for 2 h at room temperature, followed by further incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB). Finally, the slides were counterstained with Hematoxylin and mounted in an aqueous mounting medium. Appropriate positive and negative controls were stained in parallel. For negative controls, primary antibodies were replaced with PBS solution. Human cervical cancer tissues were used as a positive control.

## Evaluation of immunoreactivity

Galectin-1 protein was detected primarily in cytoplasm and partially in cytomembrane. Integrin  $\alpha 5\beta 1$  protein was observed mainly in cytoplasm and partially in nucleus. Additionally, galectin-1 and integrin  $\alpha 5\beta 1$  proteins were detected both in the tumor cells and the stroma cells of squamous cervical cancer samples. To assess all positively expressing cells, the amount of galectin-1 and integrin  $\alpha 5\beta 1$  was analyzed both in tumor cells and stromal cells. Staining evaluation was assessed by two independent observers, who were blinded to the clinical outcomes. Expression of the two markers was determined by an individual labeling score considering percent and staining intensity of positive cells [22]. Intensity of stained cells was graded semiquantitatively into four levels as following: 0 (no staining); 1 point (weak staining: light yellow); 2 points (moderate staining: yellow brown), and 3 points (strong staining: brown). The percentage was scored as following: 0 (0–5%), 1 point (6–24%), 2 points (25–49%), 3 points (50–74%), and 4 points (75–100%). Intensity and fraction of positive cell scores were multiplied for each marker, and thus, we got the immunoreactive score.

## Bioinformatics analysis

Three publicly available datasets, including TCGA database, GSE6791 and GSE9750 were used in the current study. Briefly, RNA-sequencing data, including 309 cervical samples were downloaded from TCGA database (<https://gdportal.nci.nih.gov/>) and Gene Expression Omnibus (GEO) database as referenced above were downloaded from <https://www.ncbi.nlm.nih.gov/gds/>.

Integrin  $\alpha 5$  primarily combines with integrin  $\beta 1$  subunit to form  $\alpha 5\beta 1$  heterodimer, therefore, we will analyze integrin  $\alpha 5$  and integrin  $\beta 1$  genes, respectively. There are only three cases of cervical normal tissues in TCGA database, therefore, the mRNA expression of galectin-1 and integrin  $\alpha 5\beta 1$  in cervical cancer tissues and normal tissues was determined through analysis of GEO databases. The correlation between galectin-1/integrin  $\alpha 5\beta 1$  and apoptosis-associated markers (caspase 3 and caspase 8) was investigated by using the TCGA RNA-sequencing data.

## Statistical analysis

The Kolmogorov–Smirnov test of normality was applied. Continuous variables were presented as means  $\pm$  S.D. and non-normally distributed variables were presented as median (P25– P75). Since the patient profile between

**Table 1 Clinical characteristics of cervical cancer patients between NACT responders and non-responders**

Parameter	Responders (n=17)	Non-responders (n=18)	P
Median age (years)	47 ± 8	44 ± 9	NS*
FIGO stage			NS*
IB2	8	11	
IIA2	9	7	
Differentiation			
G1	6	7	
G2	10	10	
G3	1	1	
Primary tumor size (cm)	4.53 ± 1.24	4.00 ± 1.41	NS*

\* $P > 0.05$ .

NS: no significance

the NACT responsive and non-responsive groups displayed a normal distribution, a Student's *t* test (age and tumor size) or chi-square test (FIGO stage and histological grade) was used for analysis. Protein expression between the two groups displayed a non-normal distribution and was evaluated by Wilcoxon test (comparison between pre- and postchemotherapy groups) or Mann–Whitney U test (comparison between chemotherapy response and non-response groups). The software of SPSS 16.0 (SPSS Inc, IL) was used for statistical analysis. A *P*-value less than 0.05 was considered statistically significant.

## Results

### Patient characteristics

Clinical and pathological characteristics of 35 women with squamous cervical cancer are summarized in Table 1. A CR and a PR were obtained in 5 and 12 patients, respectively, with an overall optimal response of 48.6%. Thirteen patients (37.1%) had an SD and other five patients (14.3%) showed PD. There were no significant differences between the chemotherapy responders and non-responders in age at diagnosis, the disease's FIGO stage, primary tumor size, and differentiation ( $P > 0.05$ ) (Table 1).

### Expression of galectin-1 and integrin $\alpha 5\beta 1$ status

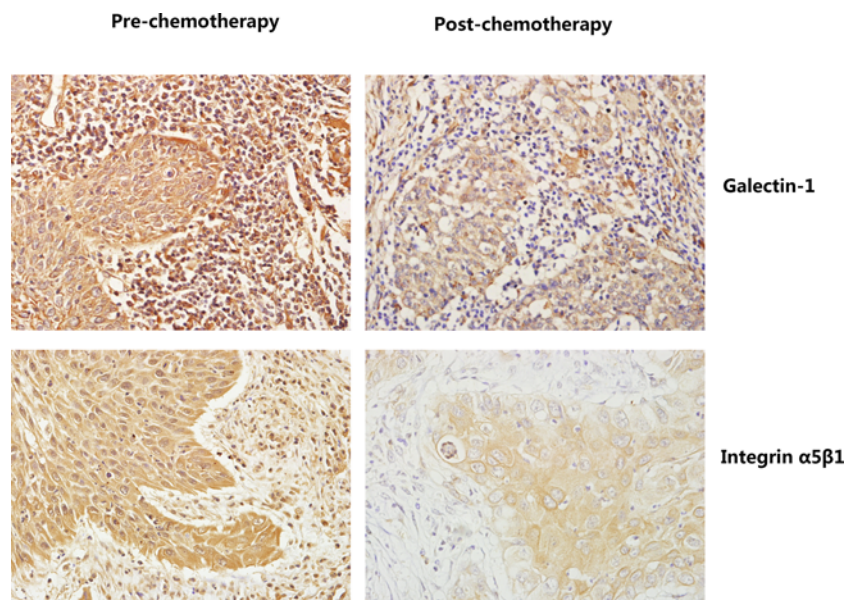
The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in 35 matched primary squamous cervical cancer biopsies before and after cisplatin-based NACT were evaluated using immunohistochemistry. As shown in Figure 1, galectin-1 and integrin  $\alpha 5\beta 1$  proteins were detected both in the tumor cells and in the stroma cells of squamous cervical cancer samples. To assess all the positively expressing cells, the amount of galectin-1 and integrin  $\alpha 5\beta 1$  was analyzed both in tumor cells and stromal cells. Galectin-1 and integrin  $\alpha 5\beta 1$  expression were detected in stroma amongst all the 70 samples. However, galectin-1 and integrin  $\alpha 5\beta 1$  protein expressed in tumor cells only in 82.86% (58/70) and 85.71% samples (60/70), respectively. Additionally, galectin-1 protein primarily localized in the cytoplasm and partially in the cytomembrane, while integrin  $\alpha 5\beta 1$  protein mainly expressed in the cytoplasm and partially in nucleus of these cells.

### Proteins expression in cervical cancers pre- and postchemotherapy

Initially, the effect of chemotherapy on the expression of galectin-1 and integrin  $\alpha 5\beta 1$  in squamous cervical cancer samples was evaluated. Prechemotherapy cervical cancer tissues consistently showed moderate or intense positive staining of galectin-1 and integrin  $\alpha 5\beta 1$  in tumor cells, while postchemotherapy tissues consistently showed weak or moderate positive staining in tumor cells (Figure 1). Using a Wilcoxon test, the expression of galectin-1 and integrin  $\alpha 5\beta 1$  in tumor cells was significantly decreased in postchemotherapy samples compared with prechemotherapy tissue samples, respectively ( $P < 0.05$ ) (Table 2). With regard to proteins' expression in stromal cells, galectin-1 and integrin  $\alpha 5\beta 1$  protein expression were also markedly down-regulated after cisplatin-based NACT when compared with their matched prechemotherapy tissues, consistent with their expression in tumor cells (Figure 1, Table 2).

Collectively, chemotherapy down-regulated the expression of galectin-1 and integrin  $\alpha 5\beta 1$  both in tumor cells and stromal cells of squamous cervical cancer, suggesting galectin-1 and integrin  $\alpha 5\beta 1$  protein were involved in cervical tumor response to chemotherapy.





**Figure 1.** The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in paired samples of squamous cervical cancer as compared with corresponding prechemotherapy and postchemotherapy (SP staining,  $\times 400$ ).

Galectin-1 and integrin  $\alpha 5\beta 1$  proteins were detected both in the tumor cells and the stroma cells of squamous cervical cancer samples. Galectin-1 protein primarily localized in the cytoplasm and partially in the cytomembrane, while integrin  $\alpha 5\beta 1$  protein mainly expressed in the cytoplasm and partially in nuclei of these cells.

**Table 2** The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in cancer cells and stromal cells of pre- and postchemotherapy cervical squamous cancer

	<i>n</i>	Galectin-1		Integrin $\alpha 5\beta 1$	
		Cancer cells	Stromal cells	Cancer cells	Stromal cells
Prechemotherapy	35	8.0 (4.00–8.00)	9.0 (7.00–12.00)	8.0 (6.50–11.00)	8.0 (6.00–11.25)
Postchemotherapy	35	4.0 (2.00–8.00)	6.0 (4.00–6.00)	4.0 (3.00–8.00)	2.0 (2.00–4.00)
Z		–3.108	–4.228	–3.617	–3.484
P		0.002*	0.00*	0.00*	0.00*

Data are expressed as median (P25–P75), \* $P < 0.05$ .

**Table 3** The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in cancer cells and stromal cells of prechemotherapy cervical cancer between chemotherapy response and non-response groups

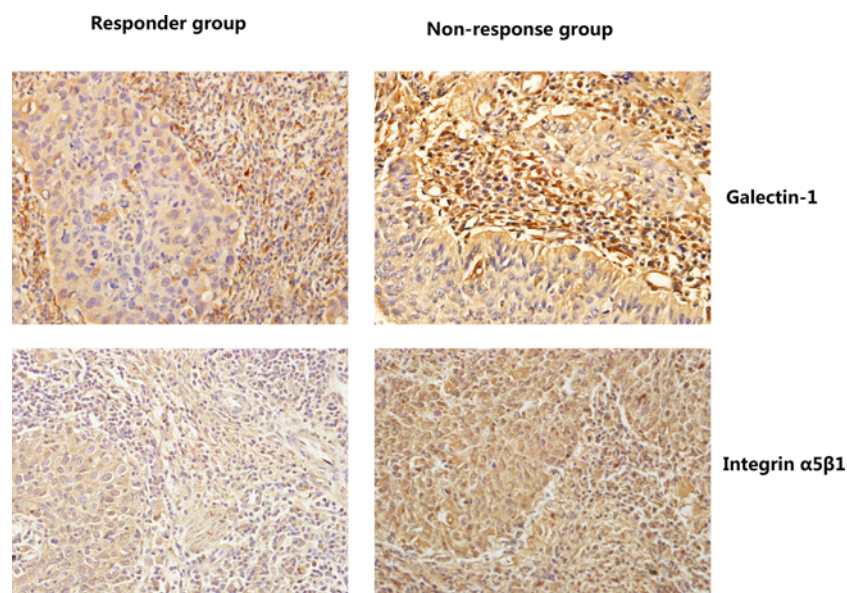
	<i>n</i>	Galectin-1		Integrin $\alpha 5\beta 1$	
		Cancer cells	Stromal cells	Cancer cells	Stromal cells
Responders	17	8.0 (4.00–8.00)	8.0 (6.00–9.00)	8.0 (6.00–8.00)	6.0 (4.00–8.00)
Non-responders	18	8.0 (4.00–8.00)	12.0 (8.00–12.00)	8.0 (8.00–12.00)	8.0 (8.00–12.00)
Z		–0.342	–2.327	–1.449	–2.795
P		0.732	0.020*	0.147	0.005*

Data are expressed as median (P25–P75), \* $P < 0.05$ .

## Proteins expression in cervical cancers between chemotherapy response and non-response groups

Then, the involvement of galectin-1 and integrin  $\alpha 5\beta 1$  proteins in predicting responses to NACT was further investigated. The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in prechemotherapy cervical cancer tissues between chemotherapy responders and non-responders was analyzed by immunohistochemistry.

As shown in Table 3, the expression of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal cells was statistically lower in the



**Figure 2.** The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in cervical squamous cancer cells and stromal cells in the chemotherapy response group and the non-response group (SP staining,  $\times 400$ )

The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal was much lower in the response group than that in the non-response group.

**Table 4** The relevance between galectin-1/integrin  $\alpha 5$ /integrin  $\beta 1$  and apoptosis associated-markers in cervical cancer

Group	n	Caspase 3		Caspase 8	
		r	P	r	P
Galectin-1	309	-0.296	0.000*	-0.211	0.000*
Integrin $\alpha 5$	309	-0.124	0.029*	-0.112	0.05*
Integrin $\beta 1$	309	0.035	0.541	0.101	0.077

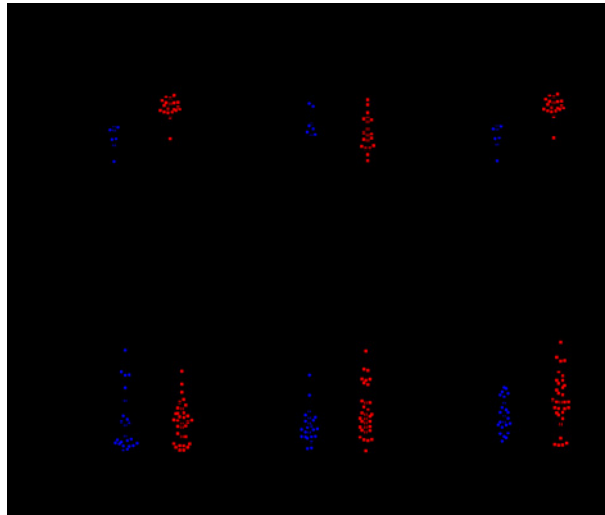
\* $P < 0.05$ ; n, numbers of patients.

response group than that in the non-response group, suggesting that patients with high levels of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal were more resistant to cisplatin-based NACT than those with low protein expression ( $P < 0.05$ , respectively) (Figure 2, Table 3). Nevertheless, the expression of galectin-1 and integrin  $\alpha 5\beta 1$  in tumor cells had no statistical difference between these two groups (Figure 2, Table 3). Thus, we suggested that the expression of galectin-1 and integrin  $\alpha 5\beta 1$  protein in stromal cells but not in tumor cells was associated with responses to NACT among squamous cervical cancer patients.

### The correlation between galectin-1/integrin $\alpha 5\beta 1$ and apoptosis-associated markers in human cervical cancer tissues

To determine the clinical relevance of the expression of galectin-1 and integrin  $\alpha 5\beta 1$ , we first analyzed the mRNA expression of galectin-1 and integrin  $\alpha 5\beta 1$  using the GEO RNA-sequencing data. As shown in Figure 3, the mRNA levels of galectin-1, integrin  $\alpha 5$ , and integrin  $\beta 1$  were much higher in cervical cancer tissues than that in normal cervical tissues.

Cisplatin-induced apoptosis was essential for the anticancer effect of cisplatin [23], we then explored the correlation between galectin-1/integrin  $\alpha 5\beta 1$  and apoptosis-associated markers. Since the sample size in GEO database was not large enough ( $n=28$  and  $n=66$ , respectively), we then analyzed their correlation by using the TCGA RNA-sequencing data ( $n=309$ ). Interestingly, a significant negative correlation was detected between galectin-1/integrin  $\alpha 5$  and caspase 3/caspase 8 (Table 4).



**Figure 3. Galectin-1 and integrin  $\alpha 5\beta 1$  are up-regulated in human cervical cancer tissues**

(a) GSE6791 data showing the expression of galectin-1, integrin  $\alpha 5$ , and integrin  $\beta 1$  in normal cervical tissues compared with cervical cancer. (b) GSE9750 data showing the expression of galectin-1, integrin  $\alpha 5$ , and integrin  $\beta 1$  in normal cervical tissues compared with cervical cancer.

## Discussion

In the current study, we reported that galectin-1 and integrin  $\alpha 5\beta 1$  expression in tumor cells and stromal cells were significantly down-regulated after NACT in patients with squamous cervical cancer. The expression of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal was much higher in patients with poor response to cisplatin-based NACT than that in the response group. These findings suggest that galectin-1 and integrin  $\alpha 5\beta 1$  may serve as potential predictive biomarkers to cisplatin-based NACT responses for patients with squamous cervical cancer. To our knowledge, this is the first study to investigate the predictive role of galectin-1 and integrin  $\alpha 5\beta 1$  in chemoresistance amongst squamous cervical cancer patients.

Galectin-1 is a  $\beta$ -galactoside-binding protein that is abundantly secreted by almost all the types of malignant tumor cells [24]. It is involved in numerous physiologic processes including cell transformation, cell proliferation, angiogenesis, cell adhesion and invasiveness, and immunosuppression [6,24]. Galectin-1 has been widely reported to be overexpressed in cervical cancer cell lines and cervical cancer samples [7,8,25]. In agreement with previous data, we found that galectin-1 mRNA was much higher in cervical cancer samples compared with normal cervical tissues. Our current study detected galectin-1 expressed both in tumor cells and stromal cells, moreover, galectin-1 accumulated more frequently in stromal cells than its in tumor cells of cervical cancer, which is consistent with previous immunohistochemical studies [7,8,25]. In addition, galectin-1 has been detected in the cytoplasm, cytomembrane, and nucleus, mainly located in the cytoplasm [25,26], which was further confirmed by the current study.

In addition to its role in tumorigenesis, the association between galectin-1 expression and chemoresistance was investigated in several types of cancer more recently. Galectin-1 expression was found to be up-regulated in cisplatin-resistant human lung adenocarcinoma A549/DDP cells compared with their parental cells [11]. Suppression of galectin-1 can sensitize lung cancer cells to cisplatin by up-regulating p38, mitogen-activated protein kinase, the extracellular signal-regulated kinase (ERK), and cyclooxygenase-2 [11,12] and down-regulating galectin-1 expression may promote sensitivity toward the proapoptotic and proautophagic drugs in glioma [27]. In contrast, increased expression of galectin-1 in epithelial ovarian cells can enhance their resistance to cisplatin by activating the H-Ras/Raf/ERK pathway and up-regulating the p21 and Bcl-2 [13]. Up-regulating galectin-1 in the hepatoma microenvironment by soluble galectin-1 during the cisplatin treatment could facilitate the chemoresistance of cancer cells via inducing autophagy [28]. In the present study, we found that the expression of galectin-1 was down-regulated both in cervical stromal cells and cervical cancer cells after chemotherapy, suggesting galectin-1 involved in chemotherapy response in cervical cancer. Moreover, patients with high levels of galectin-1 in stromal were more resistant to cisplatin-based NACT than those with low protein expression, which supported the recently highlighted roles of galectin-1 in the development of chemoresistance. It is likely that abundantly secreted galectin-1 proteins in stroma surrounding cervical tumors promoted tumor cell migration, invasion, and metastasis.

Integrin  $\alpha 5\beta 1$  is a glycoprotein adhesion molecule [14] and its alteration in many types of cancers related to the regulation of cell survival, proliferation, differentiation, apoptosis, and tumor angiogenesis [16,18,29]. Previous studies showed that integrin  $\alpha 5\beta 1$  was significantly increased in cervical cancer tissues compared with normal cervical mucosa, and higher levels of integrin  $\alpha 5\beta 1$  were related to poor histologic differentiation, lymph node metastasis, recurrence of cervical cancer, and unfavorable prognosis [19]. Consistent with this, our current study reported that mRNA levels of integrin  $\alpha 5$  as well as integrin  $\beta 1$  were much higher in cervical cancer tissues than in normal cervical tissues.

Recently, some studies showed that integrin  $\alpha 5\beta 1$  had a close association with resistance to cancer chemotherapy. Nakahara et al. [17] reported oligosaccharide changes in integrin  $\alpha 5\beta 1$  were associated with cisplatin resistance in head and neck squamous cell carcinoma cell line. Hu et al. [30] suggested that overexpression of integrin  $\alpha 5$  was strong risk factors for chemotherapeutic drug resistance in ovarian carcinoma patients. In agreement with previous studies, our data showed down-regulation of integrin  $\alpha 5\beta 1$  both in cancer cells and stromal cells following cisplatin-based NACT. Furthermore, we detected that integrin  $\alpha 5\beta 1$  expression in stromal was statistically lower in the chemotherapy response group than that in the non-response group, suggesting a higher level of integrin  $\alpha 5\beta 1$  in stromal may be a potential predictive biomarker for cisplatin chemoresistance in squamous cervical cancer patients.

Cisplatin-induced apoptosis is essential for the anticancer effect of cisplatin and inactivation of apoptosis may be the predominant cause for cisplatin resistance [23]. Caspases play critical roles in apoptosis. The activity of caspase 3, caspase 8, and caspase 9 was decreased in cisplatin-resistant cervical cancer cells [23]. In the present study, we found galectin-1 as well as integrin  $\alpha 5$  levels were negatively correlated with caspase 3 and caspase 8 levels using TCGA RNA-sequencing data, suggesting that galectin-1 and integrin  $\alpha 5$  may contribute to negative chemotherapy response via suppressing apoptosis.

In conclusion, our current study demonstrated that the expression of galectin-1 and integrin  $\alpha 5\beta 1$  in stromal can predict the responses to NACT in squamous cervical cancer. Further studies with larger cohorts are warranted to verify these results prospectively. It is also suggested from our findings that galectin-1 and integrin  $\alpha 5\beta 1$  may be implicated in the development of chemoresistance in cervical cancer via suppressing apoptosis. Our future study will further explore the role of galectin-1 and integrin  $\alpha 5\beta 1$  in chemoresistance *in vitro* and *in vivo*.

### Acknowledgements

We thank Qi Shen from the Second Affiliated Hospital of Wenzhou Medical University for Bioinformatics analysis.

### Competing interests

The authors declare that there are no competing interests associated with the manuscript.

### Funding

This work was supported by the National Natural Science Foundation of China [grant number 81602305 (to H.Z.)]; and the Science and Technology Development Funds of Wenzhou City [grant number Y20170570 (to A.C.)].

### Author contribution

X.Z. conceived and designed the research. H.Z., A.C., X.T., and S.L. performed the experiments and analyzed the data. H.Z. and S.L. wrote the manuscript. B.S. and M.C. revised the manuscript. All the authors read and approved the final manuscript.

### Abbreviations

CR, complete response; ERK, the extracellular signal-regulated kinase; FIGO, International Federation of Gynecology and Obstetrics; GEO, Gene Expression Omnibus; NACT, neoadjuvant chemotherapy; PD, progressive disease; PR, partial response; SD, stable disease; TCGA, The Cancer Genome Atlas.

### References

- 1 Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J. and Jemal, A. (2015) Global cancer statistics, 2012. *CA Cancer J. Clin.* **65**, 87–108
- 2 Siegel, R.L., Miller, K.D. and Jemal, A. (2015) Cancer statistics, 2015. *CA Cancer J. Clin.* **65**, 5–29
- 3 He, L., Wu, L., Su, G., Wei, W., Liang, L., Han, L. et al. (2014) The efficacy of neoadjuvant chemotherapy in different histological types of cervical cancer. *Gynecol. Oncol.* **134**, 419–425
- 4 Jin, L., Shen, Q., Ding, S., Jiang, W., Jiang, L. and Zhu, X. (2012) Immunohistochemical expression of Annexin A2 and S100A proteins in patients with bulky stage IB-IIA cervical cancer treated with neoadjuvant chemotherapy. *Gynecol. Oncol.* **126**, 140–146
- 5 Zou, S., Shen, Q., Hua, Y., Jiang, W., Zhang, W. and Zhu, X. (2013) Proteomic identification of neoadjuvant chemotherapy-related proteins in bulky stage IB-IIA squamous cervical cancer. *Reprod. Sci.* **20**, 1356–1364



- 6 Blanchard, H., Bum-Erdene, K., Bohari, M.H. and Yu, X. (2016) Galectin-1 inhibitors and their potential therapeutic applications: a patent review. *Expert Opin. Ther. Pat.* **26**, 537–554
- 7 Kohrenhagen, N., Volker, H.U., Kapp, M., Dietl, J. and Kammerer, U. (2006) Increased expression of galectin-1 during the progression of cervical neoplasia. *Int. J. Gynecol. Cancer* **16**, 2018–2022
- 8 Kim, H.J., Do, I.G., Jeon, H.K., Cho, Y.J., Park, Y.A., Choi, J.J. et al. (2013) Galectin 1 expression is associated with tumor invasion and metastasis in stage IB to IIA cervical cancer. *Hum Pathol.* **44**, 62–68
- 9 Li, H., Wang, Y. and Zhou, F. (2010) Effect of *ex vivo*-expanded  $\gamma\delta$ -T cells combined with galectin-1 antibody on the growth of human cervical cancer xenografts in SCID mice. *Clin. Invest. Med.* **33**, E280–E289
- 10 Huang, E.Y., Chanchien, C.C., Lin, H., Wang, C.C., Wang, C.J. and Huang, C.C. (2013) Galectin-1 is an independent prognostic factor for local recurrence and survival after definitive radiation therapy for patients with squamous cell carcinoma of the uterine cervix. *Int. J. Radiat. Oncol. Biol. Phys.* **87**, 975–982
- 11 Zhang, L., Liu, X., Tang, Z., Li, X. and Wang, G. (2016) Reversal of galectin-1 gene silencing on resistance to cisplatin in human lung adenocarcinoma A549 cells. *Biomed. Pharmacother.* **83**, 265–270
- 12 Chung, L.Y., Tang, S.J., Sun, G.H., Chou, T.Y., Yeh, T.S., Yu, S.L. et al. (2012) Galectin-1 promotes lung cancer progression and chemoresistance by upregulating p38 MAPK, ERK, and cyclooxygenase-2. *Clin. Cancer Res.* **18**, 4037–4047
- 13 Zhang, P., Zhang, P., Shi, B., Zhou, M., Jiang, H., Zhang, H. et al. (2014) Galectin-1 overexpression promotes progression and chemoresistance to cisplatin in epithelial ovarian cancer. *Cell Death Dis.* **5**, e991
- 14 Iwamoto, D.V. and Calderwood, D.A. (2015) Regulation of integrin-mediated adhesions. *Curr. Opin. Cell Biol.* **36**, 41–47
- 15 Gong, C., Yang, Z., Wu, F., Han, L., Liu, Y. and Gong, W. (2016) *miR-17* inhibits ovarian cancer cell peritoneal metastasis by targeting ITGA5 and ITGB1. *Oncol. Rep.* **36**, 2177–2183
- 16 Renner, G., Janouskova, H., Noulet, F., Koenig, V., Guerin, E., Bar, S. et al. (2016) Integrin  $\alpha 5\beta 1$  and p53 convergent pathways in the control of anti-apoptotic proteins PEA-15 and survivin in high-grade glioma. *Cell Death Differ.* **23**, 640–653
- 17 Nakahara, S., Miyoshi, E., Noda, K., Ihara, S., Gu, J., Honke, K. et al. (2003) Involvement of oligosaccharide changes in alpha5beta1 integrin in a cisplatin-resistant human squamous cell carcinoma cell line. *Mol. Cancer Ther.* **2**, 1207–1214
- 18 Ren, J., Xu, S., Guo, D., Zhang, J. and Liu, S. (2014) Increased expression of  $\alpha 5\beta 1$ -integrin is a prognostic marker for patients with gastric cancer. *Clin. Transl. Oncol.* **16**, 668–674
- 19 Wang, H.Y., Chen, Z., Wang, Z.H., Wang, H. and Huang, L.M. (2013) Prognostic significance of alpha5beta1-integrin expression in cervical cancer. *Asian Pac. J. Cancer Prev.* **14**, 3891–3895
- 20 Zhu, H., Wu, J., Zhang, W., Luo, H., Shen, Z., Cheng, H. et al. (2016) PKM2 enhances chemosensitivity to cisplatin through interaction with the mTOR pathway in cervical cancer. *Sci. Rep.* **6**, 30788
- 21 World Health Organization (1989) *Handbook for Reporting Results of Cancer Treatment*, WHO Offset Publication, Geneva
- 22 Zhu, X., Jin, L., Zou, S., Shen, Q., Jiang, W., Lin, W. et al. (2013) Immunohistochemical expression of RAGE and its ligand (S100A9) in cervical lesions. *Cell Biochem. Biophys.* **66**, 843–850
- 23 Zhu, H., Luo, H., Zhang, W., Shen, Z., Hu, X. and Zhu, X. (2016) Molecular mechanisms of cisplatin resistance in cervical cancer. *Drug Des. Devel. Ther.* **10**, 1885–1895
- 24 Astorgues-Xerri, L., Riveiro, M.E., Tijeras-Raballand, A., Serova, M., Neuzillet, C., Albert, S. et al. (2014) Unraveling galectin-1 as a novel therapeutic target for cancer. *Cancer Treat. Rev.* **40**, 307–319
- 25 Punt, S., Thijssen, V.L., Vrolijk, J., de Kroon, C.D., Gorter, A. and Jordanova, E.S. (2015) Galectin-1, -3 and -9 expression and clinical significance in squamous cervical cancer. *PLoS ONE* **10**, e0129119
- 26 Clerch, L.B., Whitney, P., Hass, M., Brew, K., Miller, T., Werner, R. et al. (1988) Sequence of a full-length cDNA for rat lung beta-galactoside-binding protein: primary and secondary structure of the lectin. *Biochemistry* **27**, 692–699
- 27 Le Mercier, M., Lefranc, F., Mijatovic, T., Debeir, O., Haibe-Kains, B., Bontempi, G., Decaestecker, C. et al. (2008) Evidence of galectin-1 involvement in glioma chemoresistance. *Toxicol. Appl. Pharmacol.* **229**, 172–183
- 28 Su, Y.C., Davuluri, G.V., Chen, C.H., Shiau, D.C., Chen, C.C., Chen, C.L. et al. (2016) Galectin-1-induced autophagy facilitates cisplatin resistance of hepatocellular carcinoma. *PLoS ONE* **11**, e0148408
- 29 Yang, B., Gao, J., Rao, Z. and Shen, Q. (2013) Clinicopathological and prognostic significance of  $\alpha 5\beta 1$ -integrin and MMP-14 expressions in colorectal cancer. *Neoplasma* **60**, 254–261
- 30 Hu, Z., Gao, S., Gao, J., Hou, R., Liu, C., Liu, J. et al. (2012) Elevated levels of Lewis y and integrin  $\alpha 5\beta 1$  correlate with chemotherapeutic drug resistance in epithelial ovarian carcinoma. *Int. J. Mol. Sci.* **13**, 15588–15600