Original Article
Galectin-1 and DNMT1 are highly expressed and related to the prognoses of patients with cervical cancer

Xiaomin Guo1, Chunyan Yang2, Hongying Zhao3, Xingjun Wu4, Yanling Dai5

1School of Nursing, Chuxiong Medical and Pharmaceutical College, Chuxiong City, Yunnan Province, China; 2Department of Pharmacy, Chuxiong Medical and Pharmaceutical College, Chuxiong City, Yunnan Province, China; 3Department of Tumor and Hematology, The First People’s Hospital, Honghe Prefecture, Mengzi City, Yunnan Province, China; 4Qujing Medical College, Qujing City, Yunnan Province, China; 5Department of Laboratory, Chuxiong Medical and Pharmaceutical College, Chuxiong City, Yunnan Province, China

Received October 23, 2019; Accepted November 21, 2019; Epub March 15, 2020; Published March 30, 2020

Abstract: Objective: To explore the expressions of galectin-1 and human DNA methyltransferase 1 (DNMT1) in cervical cancer tissues and normal cervical tissues and their clinical significance. Method: Eighty-four patients with cervical cancer were randomly selected. 84 samples of cancer tissues and their para-carcinoma tissues were analyzed with regard to the following parameters: Galectin-1 and DNMT1 mRNA analyzed by RT-PCR, and galectin-1 and DNMT1 protein by western blotting. The relationships between the expressions of galectin-1 and DNMT1 protein, the clinicopathological factors, and the prognoses were analyzed. A correlation analysis was performed between the galectin-1 and DNMT1 protein expressions in the cervical cancer tissues. Result: The expressions of galectin-1 and DNMT1 mRNA and protein in the cervical cancer tissues were higher than they were in the para-carcinoma tissues (P<0.05). The expression of galectin-1 protein was correlated with pathological stage, lymph node metastasis, high-risk HPV infection, and pathological differentiation (P<0.05), but not with age or pathological type (P>0.05). The expression of DNMT1 protein was correlated with pathological stage, lymph node metastasis, high-risk HPV infection, and pathological differentiation (P<0.05), but not with age or pathological type (P>0.05). The 3-year survival of subjects with a high expression of galectin-1 or DNMT1 protein was significantly lower than it was in patients with low expressions (P<0.05). There was a positive correlation between the expressions of galectin-1 and DNMT1 mRNA in cervical cancer tissues (r=0.599, P<0.001). The expressions of galectin-1 and DNMT1 protein in cervical cancer tissues are also positively correlated (r=0.617, P<0.001). Conclusion: Galectin-1 and DNMT1 are highly expressed in cervical cancer tissues, and their expressions are closely related to the pathological stage, lymph node metastasis, high-risk HPV infection, pathological differentiation, and the prognosis of patients with cervical cancer, and the expressions may be used as a predictor of disease progression and prognosis.

Keywords: Cervical cancer, galectin-1 (Gal-1), human DNA methyltransferase 1 (DNMT1), HR-HPV

Introduction

Cervical cancer is the most common malignancy of the female reproductive system. It has a high incidence, and in recent years, its incidence is on the rise, and the age of onset is getting lower [1, 2]. In recent years, many aspects of cervical cancer have been studied [3, 4]. Although studies [5] have shown that the incidence of cervical cancer is closely related to the persistent infection of high-risk human papilloma viruses (HR-HPV), the specific pathogenesis remains unclear.

Galectin-1 (Gal-1) is a member of the galectin family and is widely distributed [6]. Studies have shown that Gal-1 is closely related to the occurrence, development, and metastasis of stomach cancer, and it is considered crucial to the development of tumors [7]. Other studies have shown that Gal-1 can specifically affect biological processes such as cell proliferation and apoptosis by binding to β-galactoside [8]. However, there is also one study which explored the role of galectins, especially galectin-1, in cervical cancer, and it found that targeting Gal-1 may improve the local control of cervical cancer [9].

In the study of malignant tumors, DNA methylation is an epigenetic regulation mechanism that has received much attention in recent years,
and DNA methyltransferase (DNMT) is a key enzyme that catalyzes DNA methylation modification [10]. During the occurrence and development of malignant tumors, DNA methylation modification can inhibit the expression of tumor suppressor factors and pro-apoptotic factors, thus contributing to the growth of tumor cells [11]. It has been reported that DNMT1 is abnormally expressed in cervical cancer [12]. DNMT1 acts as a subtype of DNMTs and acts primarily on the maintenance of methyltransferases of hemimethylated DNA. Also, research has found that DNMT1 is significantly associated with poor cervical cancer survival outcomes [13].

Therefore, in order to further explore whether the expressions of Gal-1 and DNMT1 in cervical cancer play a role in the development of the disease, the expressions of Gal-1 and DNMT1 in cervical cancer tissues and normal tissues and their clinical significance are discussed.

Materials and methods

General information

84 patients with cervical cancer who were admitted to our hospital and underwent surgery were selected as the study cohort. During the operation, 84 samples of cancer tissue and their para-carcinoma tissue were obtained (All tissues were stored in liquid nitrogen immediately after intraoperative resection) The patients had a mean age of (47.33±5.19) years. According to the pathological classification, there were 41 cases of adenocarcinoma and 43 cases of squamous cell carcinoma. There were 45 patients in pathological stages I and II and 39 patients in stage III. There were 44 patients with lymph node metastasis and 40 patients without lymph node metastasis.

Exclusion and inclusion criteria: Inclusion criteria: Patients diagnosed with cervical cancer through a pathological diagnosis; patients with a predicted survival time > 3 months.

Exclusion criteria: Patients with insufficient pathology for diagnosis; patients with other types of malignant tumors; patients who received preoperative chemoradiotherapy; patients with severe liver or kidney dysfunction; patients with cognitive or communication disorders; patients with poor compliance. All patients and their families agreed to participate in the experiment and signed the informed consent forms. This experiment was approved by the hospital ethics committee.

Experimental reagents and materials

Gal-1, DNMT1, and β-actin rabbit anti-human polyclonal antibody were purchased from Biyuntian Technology Co., Ltd. Gal-1 mRNA, and DNMT1 mRNA primers were purchased from Shanghai Jima Company. Trizol reagent was purchased from Invitrogen, USA. The real-time quantitative PCR instrument was purchased from BioRad, USA. The qPCR kit and the minScript reverse transcription kit were purchased from Dalian TaKaRa.

Quantification of the Gal-1 and DNMT1 mRNA expressions in the cervical cancer and para-carcinoma tissues using RT-PCR

The surgical cancer and para-carcinoma tissues excised in the operations were removed from the liquid nitrogen tank. Total RNA was extracted using the Trizol reagent. The purity and concentration of the RNA were then determined using an ultraviolet spectrophotometer. The reverse transcription of the cDNA was performed by taking 1 μg of total RNA according to the kit’s instructions. The transcribed cDNA was used for PCR amplification. The PCR amplification system was configured according to the manufacturer’s instructions. The PCR took β-actin as the internal reference, and the primer sequence is shown in Table 1. The PCR reaction parameters detected by gal-1 mRNA were as follows: It was pre-denatured at 94°C for 5 min, then denatured at 95°C for 1 min, annealed at 60°C for 1 min, and at 72°C for 50 s, for a total of 35 cycles. The PCR reaction parameters of the DNMT1 mRNA detection were as follows: It was pre-denatured at 95°C for 5 min, then denatured at 95°C for 30 s, annealed at 60°C for 30 s, for a total of 40 cycles, and finally at 72°C for 5 min. The experiment was repeated 3 times with β-actin as the
Differences in the expressions of galectin-1 and human DNA methyltransferase 1


Internal control. The relative quantification of the target genes was calculated using $2^{\Delta\Delta C_{t}}$.

**Western blotting was used to quantify the expressions of the Gal-1 and DNMT1 proteins in the cervical cancer tissues and para-carcinoma tissues**

First, the cervical cancer and para-carcinoma tissues were taken out from the liquid nitrogen tank, and the RIPA lysate was added to the tissue for lysis on ice. The lysed tissue was denatured in a water bath at 100°C for 15 min to denature the protein, and then the total protein was collected. The protein was then separated by 10% SDS-PAGE and transferred to a PVDF membrane. It was then blocked with 5% skim milk for 1 h at room temperature. Then, Gal-1 (1:1000), β-actin (1:500), and DNMT1 (1:1000) primary antibodies were added and allowed to stand overnight at 4°C. The secondary antibody was then added for incubation, and finally the blot was finished with the ECL developer. A grayscale analysis was performed with Image LabTM software. The relative expression levels of the protein = band gray value of the target protein/band gray value of the β-Actin protein.

**Outcome measures**

(1) The expression levels of Gal-1 and DNMT1 mRNA and the protein in the cervical cancer tissues and para-carcinoma tissues were compared. (2) The relationship between the expression levels of the Gal-1 and DNMT1 proteins and the clinicopathological characteristics of the patients with cervical cancer were analyzed. (3) The relationship between the expression levels of Gal-1 and the DNMT1 proteins and the prognosis of the patients with cervical cancer was analyzed. The patients were followed up every 6 months for three consecutive years. The percentage of patients still alive for this given period of time was recorded. (4) A correlation analysis was performed between the Gal-1 and DNMT1 proteins and the mRNAs in the cervical cancer tissue.

**Statistical methods**

In this experiment, SPSS 19.0 statistical software (Beijing Wangshu Times Technology Co., Ltd.) was used for the statistical analysis of the experimental data. The counting data between the two groups were tested using $X^2$ tests. The measurement data were analyzed by independent t tests and expressed as the means ± standard deviations. The survival curve was estimated using a nonparametric Kaplan-Meier curve and analyzed using a log rank test. The correlation analysis was performed using a Pearson analysis. P<0.05 was considered statistically significant.

**Results**

- The expression of the Gal-1 protein was higher in patients with advanced pathological stages, Figure 1. The expression levels of galectin-1 and DNMT1 mRNA in the cervical cancer and adjacent tissues. The expressions of the Gal-1 and DNMT1 mRNA in the cervical cancer tissues were higher than they were in the para-carcinoma tissues (P<0.05). Note: * indicated P<0.05.

**Lal-1 and DNMT1 protein expressions across the clinicopathological factors in patients with cervical cancer**

The expressions of Gal-1 mRNA and protein in the cervical cancer tissues were (2.13±0.11) and (5.27±0.15), respectively. The expressions of DNMT1 mRNA and protein were (2.36±0.25) and (6.73±0.22), respectively. The expressions of Gal-1 mRNA and protein in the para-carcinoma tissue were (0.98±0.08) and (2.54±0.14), respectively. The expressions of DNMT1 mRNA and protein were (1.03±0.16) and (2.71±0.36), respectively. The expressions of Gal-1 and the DNMT1 mRNA and protein in the cervical cancer tissues were higher than they were in the para-carcinoma tissues (P<0.05) (Figures 1, 2).

**Lal-1 and DNMT1 protein expressions across the clinicopathological factors in patients with cervical cancer**

The expression of the Gal-1 protein was higher in patients with advanced pathological stages,
Differences in the expressions of galectin-1 and human DNA methyltransferase 1

lymph node metastasis, high-risk HPV infection, and low pathological differentiation (P<0.05) (P>0.05). The expression of the DNMT1 protein was correlated with pathological stage, lymph node metastasis, high-risk HPV infection and pathological differentiation (P<0.05) (Table 2).

The relationship between the expression levels of the Gal-1 and DNMT1 proteins and the prognoses of patients with cervical cancer

According to the mean expressions of the Gal-1 and DNMT1 proteins, the patients were divided into a high expression group (>5.27) and a low expression group (≤5.27) of the Gal-1 protein, and a high expression group (>6.73) and a low expression group (≤6.73) of the DNMT1 protein. There were 43 patients with high expressions of Gal-1 protein and 41 patients with low expressions. There were 44 patients with high expressions of DNMT1 protein and 40 patients with low expressions. The 3-year survival rates of the patients with high expressions of the Gal-1 protein and the low expression group were 41.86% and 63.41%, respectively. The 3-year survival rates of patients with high expressions and low expressions of the DNMT1 protein were 38.64% and 67.50%, respectively. The 3-year survival rate in patients with high expressions of the Gal-1 and DNMT1 proteins was significantly lower than it was in patients with low expressions (P<0.05) (Table 3; Figures 3, 4).

The correlation between the galectin-1 and DNMT1 mRNA and the protein expressions in the cervical cancer tissues

There was a positive correlation between the expressions of galectin-1 and DNMT1 mRNA (R=0.599, P<0.001) and between the expressions of galectin-1 and DNMT1 protein (R=0.617, P<0.001) in the cervical cancer tissues (Figures 5, 6).

Discussion

Gal-1 protein is a member of the galectin family and is encoded by the LGALS1 gene. It is located on the 22q13.1 chromosome and plays an important role in tumor invasion and metastasis [14]. It has an important influence on cell proliferation and apoptosis by binding the layers of adhesion cells, the extracellular matrix, and other targeting molecules [15]. Previous studies found that the expression of Gal-1 in malignant tumor tissues is generally up-regulated, and its expression is considered to be related to malignancy, invasion, and metastasis [16]. In our study, the expressions of Gal-1 in the cervical cancer tissues and para-carcinoma tissues were also compared. The results showed that the expression of the Gal-1 mRNA or the Gal-1 protein in the cervical cancer tissues was higher than it was in the para-carcinoma tissues (P<0.05), suggesting that Gal-1 is also highly expressed in cervical cancer tissue. The relationships between the expression of Gal-1 and the clinicopathological factors and the patients’ prognoses were also determined.

Figure 2. The expression levels of the galectin-1 and DNMT1 proteins in the cervical cancer and adjacent tissues. The expressions of Gal-1 and DNMT1 in the cervical cancer tissues were higher than they were in the para-carcinoma tissues (P<0.05). Note: * indicated P<0.05.
Differences in the expressions of galectin-1 and human DNA methyltransferase 1

Table 2. The relationship between galectin-1 and DNMT1 protein expression and different clinicopathological features in cervical cancer

<table>
<thead>
<tr>
<th>Factors</th>
<th>n</th>
<th>galectin-1 t</th>
<th>P</th>
<th>DNMT1 t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤47</td>
<td>38</td>
<td>5.25±0.16</td>
<td>0.327</td>
<td>6.69±0.27</td>
<td>0.55</td>
</tr>
<tr>
<td>&gt;47</td>
<td>46</td>
<td>5.26±0.21</td>
<td>0.744</td>
<td>6.72±0.23</td>
<td>0.584</td>
</tr>
<tr>
<td>Pathological stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>45</td>
<td>3.69±0.33</td>
<td>34.71</td>
<td>4.15±0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>I</td>
<td>39</td>
<td>7.52±0.61</td>
<td>82.26</td>
<td>8.91±0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>9.33±0.24</td>
<td>91.41</td>
<td>8.17±0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
<td>3.05±0.38</td>
<td>73.61</td>
<td>4.35±0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>high-risk HPV infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71</td>
<td>5.89±1.13</td>
<td>3.257</td>
<td>7.32±1.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>4.86±0.25</td>
<td></td>
<td>3.04±0.41</td>
<td>0.398</td>
</tr>
<tr>
<td>Pathological typing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>43</td>
<td>5.22±0.16</td>
<td>1.616</td>
<td>6.71±0.19</td>
<td>0.849</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>41</td>
<td>5.28±0.18</td>
<td></td>
<td>6.75±0.24</td>
<td>0.398</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highly</td>
<td>31</td>
<td>4.19±0.33</td>
<td>732.1</td>
<td>4.37±0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate</td>
<td>29</td>
<td>5.37±0.38</td>
<td></td>
<td>6.84±0.35</td>
<td>0.398</td>
</tr>
<tr>
<td>Low</td>
<td>24</td>
<td>7.59±0.25</td>
<td></td>
<td>9.66±0.31</td>
<td>0.398</td>
</tr>
</tbody>
</table>

Table 3. The relationship between the expression levels of the galectin-1 and DNMT1 proteins and the prognoses of patients with cervical cancer [n (%)]

<table>
<thead>
<tr>
<th>Factor</th>
<th>3-year survival rate</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>galectin-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High expression group n=43</td>
<td>18 (41.86)</td>
<td>3.909</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Low expression group n=41</td>
<td>26 (63.41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNMT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High expression group n=44</td>
<td>17 (38.64)</td>
<td>6.998</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Low expression group n=40</td>
<td>27 (67.50)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results showed that the expression of the Gal-1 protein correlated with pathological stage, lymph node metastasis, high-risk HPV infection, and pathological differentiation (P<0.05). This suggests that Gal-1 may play a role in the development and metastasis of cervical cancer. Previous studies [17] indicated that the content of the Gal-1 protein is closely related to the adverse prognostic factors in patients with malignant tumors. Previous studies [18] have explained one of the mechanisms by which Gal-1 promotes tumor cell proliferation and invasion. That is, it can promote tumor angiogenesis by inhibiting the JAK2 and RET signaling pathways and by up-regulating the expression level of vascular endothelial growth factor receptor 3. This also explained our conclusions.

With regard to the occurrence and development of malignant tumors, epigenetics has also become a hot topic in recent years. This means that a gene or protein has undergone a transformation that does not involve a DNA sequence and that can be stably inherited to the next generation of genetic phenomena [19]. At present, the most popular research on epigenetics is DNA methylation. It has been proven [20] that the most common modification in the early stage of tumors is the general hypomethylation of the repeat region of the tumor suppressor gene promoter. The process of methylation is generally accomplished by the catalysis of DNA methyltransferases, and DNMT1 is one of the macromolecular enzymes [21]. There have also been studies [22] showing that DNMT1 is highly expressed in malignant tumor tissues, so it is believed to have an important impact on the progression of cancer. In our study, DNMT1 mRNA and protein were found to be significantly higher in cervical can-
Differences in the expressions of galectin-1 and human DNA methyltransferase 1

The expression of DNMT1 protein was also associated with pathological stage, lymph node metastasis, high-risk HPV infection, and pathological differentiation (P<0.05), and the 3-year survival rate of patients with high DNMT1 protein expressions was significantly lower than it was in patients with low expressions (P<0.05). This suggests that DNMT1 may be a predictor of poor prognosis in patients with cervical cancer.

Previous studies [23] found that its expression in cervical cancer tissue is significantly higher than in normal cervical tissue, but there is no significant correlation between the DNMT1 expression level, tumor stage, or lymph node metastasis. This has certain discrepancies with our conclusions. It is speculated that this may be due to the heterogeneity of the included patients, which will be further confirmed in subsequent studies. However, studies [24] have concluded that high-risk HPV infection is closely related to the expression of DNMT1. For example, some studies [25] have found that the HPV16 oncoproteins E6 and E7 activate and up-regulate DNMT expression. Furthermore, E6 mainly affects DNMT by inhibiting the P53 gene, which affects the expression of DNMT by inhibiting the pRB/E2F pathway that regulates the DNMT1 promoter. Other studies [26] ex-
Differences in the expressions of galectin-1 and human DNA methyltransferase 1

explored the transfection of human primitive foreskin keratinocytes into free high-risk HPV. It was found that the expression of DNMT1 was increased in the transfected cells, and the methylation of the cell gene was also changed, which was also observed in cervical cancer. All of the above studies confirm and explain our conclusions.

Finally, a correlation analysis was performed between the relationship between the Gal-1 and DNMT1 expressions. The results suggest that the Gal-1 and DNMT1 mRNA protein expressions are positively correlated, suggesting that Gal-1 and DNMT1 may play a complementary role in the development of cervical cancer. However, there has been no relevant research on the relationship between Gal-1 and DNMT1.

In summary, galectin-1 and DNMT1 are highly expressed in cervical cancer tissues. These high expressions are closely related to the pathological stage, lymph node metastasis, high-risk HPV infection, pathological differentiation, and the prognosis of patients with cervical cancer and may be used as a predictor of disease progression and prognosis. However, there are certain deficiencies in our research. For example, no further independent risk factors for cervical cancer were analyzed, and no corresponding in vitro studies were performed on the relationship between Gal-1 and DNMT1. But this will be an important direction for further exploration in subsequent experiments. The relationship between the expressions of Gal-1 and DNMT1 and the clinicopathological factors of patients with cervical cancer will be further confirmed. The roles of Gal-1 and DNMT1 in cervical cancer and their correlation are also expected to be further explored by researchers in the future.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

None.

Address correspondence to: Yanling Dai, Department of Laboratory, Chuxiong Medical and Pharmaceutical College, Donggua Town, Chuxiong 675000, Yunnan, China. Tel: +86-0878-3875484; E-mail: kz87dr1@163.com

References

Differences in the expressions of galectin-1 and human DNA methyltransferase 1


