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Original contribution

Galectin 1 expression is associated with tumor invasion and metastasis in stage IB to IIA cervical cancer☆

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Summary Galectin 1 is a 14-kd laminin-binding lectin involved in important biologic mechanisms of tumors, including neoplastic transformation, cell survival, angiogenesis, cell proliferation, and metastasis. In this study, we investigated the role of galectin 1 in cell survival and metastasis in cervical cancer. The expression of galectin 1 was determined in 73 formalin-fixed, paraffin-embedded cervical cancer tissues using an immunohistochemical method and compared with clinicopathologic risk factors for recurrence after surgery. To evaluate the role of galectin 1 in cell proliferation and invasion, we performed proliferation and invasion assays with galectin 1 small interfering RNA (siRNA) using cervical cancer cell lines, including HeLa and SiHa cells. Immunohistochemical analysis revealed that galectin 1 expression was found in most peritumoral stroma samples (72/73; 98.6%). Galectin 1 expression was significantly correlated with the depth of invasion in the cervix ($P = .015$) and lymph node metastasis ($P = .045$) on univariate analysis. When progression-free survival of all of the patients studied was analyzed based upon galectin 1 expression, galectin 1 expression was not correlated with progression-free survival ($P = .32$). Down-regulation of galectin 1 using small interfering RNA resulted in the inhibition of cell growth and proliferation of HeLa and SiHa cells. Moreover, the ability of cells to invade was significantly reduced by galectin 1 small interfering RNA. Our results revealed that high galectin 1 expression in peritumoral stroma was significantly correlated with depth of invasion in cervical lesions and lymph node metastasis of cervical cancer and that galectin 1 may be functionally involved in cell proliferation and invasion.

1. Introduction

Cervical cancer is the result of a multistep process that involves transformation of the normal cervical epithelium to a preneoplastic cervical intraepithelial neoplasia that is subsequently transformed to invasive cervical cancer [1,2]. Although the incidence and mortality of invasive cervical

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cancer have steadily decreased [3], cervical cancer is still the second most common cancer of women worldwide and one of the most lethal female malignancies in developing countries [4]. Moreover, patients at high risk for recurrence after radical surgery for early-stage cervical cancer require potent adjuvant therapy, including radiation, chemotherapy, or both, to prevent disease recurrence [5]. Therefore, early identification of high-risk factors that require adjuvant therapy and the development of new therapeutic strategies are quite important to improve survival in these patients.

Galectins are a family of proteins characterized by their affinity for β-galactoside and sequence similarities in the carbohydrate-recognition domain (CRD) [6]. To date, 15 mammalian galectins have been identified, which can be subdivided into those that have 1 CRD and those that have 2 CRDs; galectin 3, a 1-CRD galectin, is unique in that it contains unusual tandem repeats of short amino acid stretches fused onto the CRD [7,8]. Galectins play a number of important roles in cancer in that they contribute to neoplastic transformation, tumor cell survival, angiogenesis, and tumor metastasis. They can modulate immune and inflammatory responses and might play a key role in helping tumors to escape immune surveillance [9]. Galectin 1 (Gal-1) behaves as a monomer of 14.5 kd that can dimerize under certain circumstances. Each monomer is composed of a CRD that recognizes a wide range of glycoproteins and glycolipids [8]. Gal-1 is involved in a variety of activities, including cell proliferation, cell migration, cell adhesion, inflammation, and immune responses [10-12]. Furthermore, it also plays a role in tumor progression of many other carcinomas, such as colorectal, nasopharyngeal, ovarian, and prostate carcinomas [13-16].

For cervical cancer, different studies have linked the galectins expression to host-parasite interaction [17], neoplastic transformation [18-20], sensitivity to concurrent chemoradiotherapy [21,22], tumorigenesis [23,24], and cell growth [25]. However, little is known about the role of Gal-1 in invasive cervical cancer, especially in terms of tumor proliferation, invasion, or metastasis. In this study, therefore, we investigated the role of Gal-1 in cell proliferation, invasion, and metastasis in cervical cancer.

2. Materials and methods

2.1. Tumor samples

A total of 83 paraffin-embedded, formalin-fixed tissue specimens were used in this study. These included 73 invasive cervical cancers from patients who underwent type III radical hysterectomy and 10 normal cervical specimens, which were obtained as controls from hysterectomy specimens of women diagnosed with uterine leiomyoma without cervical pathology. All available hematoxylin and eosin stain slides were reviewed by a pathologist. Of 73 invasive cancers, 27 patients had pelvic lymph node (LN) metastasis. All specimens were obtained from the Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea. All samples were collected according to the Institutional Review Board of Samsung Medical Center (IRB no.: 2009-09-002-002).

All patients with cervical cancer were staged clinically and underwent type III radical hysterectomy. We only enrolled patients with early-stage cervical cancer who were candidates for surgery because pathologic findings provide the most accurate information about tumor progression, including depth of tumor invasion and LN metastasis. After surgery, we categorized the patients according to pathologic risk factors into a high-risk group and an intermediate-risk group. The high-risk group included patients with positive pelvic LNs, microscopic parametrial invasion, or positive resection margins [5]. The intermediate-risk group included patients with stromal invasion of more than half of the cervix, lymphovascular space invasion, and a large tumor size (the largest diameter was $\geq$4 cm) [5]. Patients with more than 1 of the 3 high-risk factors received adjuvant pelvic radiotherapy (RT) and concurrent chemotherapy (cisplatin 40 mg/m^2 per week) [5,26]. Patients who had 3 intermediate-risk factors received adjuvant RT alone. The RT consisted of 1.8 Gy on days 1 to 5 of each week for a total 28 fractions, with a total dose of 50.4 Gy of external radiation to a standard pelvic field. Patients had follow-up examinations approximately every 3 months for the first 2 years, every 6 months for the next 3 years, and every year thereafter. We defined the disease-free interval as the time from the initial treatment to relapse noted on imaging or the last follow-up visit.

2.2. Immunohistochemistry

Immunohistochemical studies were carried out on formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections. The primary antibodies used were rabbit polyclonal Gal-1 antibodies (a gift from Dr Sabine Andre, Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Munich, Germany). Tissue sections were deparaffinized 3 times in xylene for a total of 15 minutes and subsequently rehydrated. Immunostaining for Gal-1 was performed using a Bond-Max automated immunostainer (Leica Biosystems, Melbourne, Australia) and the Bond Polymer Refine detection kit (DS9800, Vision Biosystems, Melbourne, Australia). In brief, antigen retrieval was carried out at 97°C for 20 minutes in ER1 buffer. After blocking the endogenous peroxidase activity with 3% hydrogen peroxidase for 10 minutes, the primary antibody incubation was carried out for 15 minutes at room temperature at an antibody dilution of 1:200. Staining for Gal-1 was considered positive when tumor or stromal cells showed cytoplasmic reactivity. Negative controls (substituting Tris-buffered saline for primary antibody) were run simultaneously. Two