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Role of laminin 332 in lymph node metastasis of papillary thyroid carcinoma

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ABSTRACT

Objective: The invasiveness of papillary thyroid carcinoma (PTC), including the occurrence of cervical lymph node metastasis, is the main determining factors contributing to recurrence and poor prognosis. Laminin 332 is a glycoprotein involved in cell migration and cancer cell invasion into surrounding tissues and is therefore related to poor prognosis in many cancers. Here, we investigated the expression and role of laminin 332 in PTC and examine the possibility that laminin 332 could be involved in the invasiveness of PTC.

Methods: Laminin 332 expression was determined by immunohistochemical staining in all 40 patients. The correlations between laminin 332 expression and clinical factors were investigated. We examined the expression of the laminin 332 γ2 chain using reverse transcription polymerase chain reaction and western blotting in PTC cells and determined the relationship between the expression of laminin 332 and the invasiveness of these cell lines using cell invasion assays.

Results: Laminin 332 was expressed specifically within tumor tissue. The frequency of laminin 332 γ2 chain expression was significantly correlated with cervical lymph node metastasis ($p = 0.003$). Invasiveness increased as the expression of laminin 332 γ2 increased in the tested PTC cell lines.

Conclusion: Laminin 332 expression may be a useful marker for predicting lymph node metastasis in papillary thyroid carcinoma, and could increase the ability of cancer cells to invade, which would influence the prognosis of patients with PTC.

1. Introduction

Thyroid carcinoma is the most common endocrine cancer and has a high incidence relative to other types of cancer in humans [1]. Among thyroid malignancies, papillary thyroid carcinoma (PTC) is the most common, accounting for 70–90% of all well-differentiated thyroid malignancies [2]. Patients with PTC generally have a good prognosis, and the majority of patients who undergo appropriate treatment display excellent outcomes [1,3]. Thus, there is controversy regarding potential overdiagnosis and excessive interventions in patients with PTC [4]. However, clinician agree that PTC should be aggressively treated when the prognosis is poor. The factors associated with poor prognosis include age, extracapsular extension, lymph node metastasis (LNM), and poorly differentiated cell type [2]. In addition, various molecular, biological, and genetic factors are being studied for their possible association with prognosis in patients with PTC. Primary LNM occurs in

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approximately 30–40% of adult patients [5]. Additionally, LNM, a risk factor for local and regional recurrence, affects the treatment modality employed; ensuring optimal combined treatment for LNM requires a better understanding of the molecular mechanisms underlying its development. However, no definite predictor of cervical LNM in patients with PTC has been determined [1].

Laminin is a glycoprotein produced by endothelial and epithelial cells and localized in the basement membrane. Laminin plays roles in numerous cellular functions, including cell migration, adhesion, and differentiation [6]. Structurally, laminins consist of three chains (α, β, and γ) and are named according to their chain composition [7]. Thus far, 16 laminins have been characterized. Laminin 322 (previously known as laminin 5) is a 460-kDa molecule that is composed of α3, β3, and γ2 chains. *LAMC2*, the human gene encoding the γ2 chain, is located on chromosome 1q5-q31. Laminin 332 functions as an adhesive molecule in mature normal tissue and mediates the migration of epithelial cells during wound repair [8]. Additionally, laminin 332 appears to play an important role in tumor invasion, and recent studies have supported the hypothesis that laminin 332 expression in cancer cells promotes cells growth, invasion, and metastasis [9]. Moreover, expression of laminin 332 is related to poor prognosis in breast, colon, and pancreatic cancer [10–12]. In the present study, we aimed to identify the relationship between the expression of laminin 332 and LNM in PTC. Furthermore, we investigated the association between laminin 322 expression and invasiveness in PTC using PTC cell lines.

2. Patients and methods

2.1. Patients

PTC samples were obtained randomly from the surgical pathology archive of Ansan Hospital, Korea University Medical Center between May 2006 and December 2008. Study procedures were approved by the Institutional Review Board of Korea University Medical Center. The present study involved 40 patients with well-differentiated papillary thyroid carcinoma (seven men and 33 women) who underwent thyroidectomy with central compartment neck dissection at the Department of ENT, Ansan Hospital, Korea University Medical Center. The patients' ages ranged from 25 to 82 years (mean, 48.3 years). Among the 40 patients, 20 had LNM. LNM was evaluated by pathologic finding. None of the patients received pre-operative chemotherapy or radiotherapy, and none had synchronous or metachronous cancers in other organs.

2.2. Immunohistochemistry

Tumor samples were fixed with 10% formaldehyde solution, embedded in paraffin, and sectioned into 4-μm-thick slices. Tissue sections were deparaffinized with xylene, incubated with 0.3% hydrogen peroxide in methanol, and treated with protease XXIV (Sigma, St. Louis, MO, USA). The sections were then treated with blocking solution from a Cap-plus Detection Kit (Invitrogen, Carlsbad, CA, USA). Blocked sections were incubated overnight with monoclonal anti-laminin 332 γ2 antibodies (D4B5; 1:200; Chemicon Int., Germany) at 4 °C, washed with Tris-buffered saline (TBS), and incubated with secondary antibodies and streptavidin-horseradish peroxidase (HRP) from the Cap-plus Detection Kit. The sections were then processed with 3,3-diaminobenzidine (DAB) from the Cap-plus Detection Kit. Thereafter, sections were rinsed briefly in water, counterstained with Meyer's hematoxylin (Sigma), and mounted.

Scoring and interpretation of immunohistochemical results was independently performed by two pathologists and two otolaryngology-head and neck surgeons blinded to the clinical outcomes. The immunohistochemical score for the expression of laminin 332 was assigned based on the percentage of stained tumor cells, as follows: 0, <5%; 1+, 5–25%; 2+, 26–50%; and 3+, >50%. The correlations of laminin 332 expression with clinical factors, such as tumor size, multiplicity, and LNM, were also investigated.

2.3. Cell culture

SNU-790 cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea), and BHP10-3 and BCPAP cells were kindly provided by Professor Soon Hyun Ahn (Seoul National University, Korea); all three of these cell lines are PTC cell lines. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and L-glutamine (300 mg/L) [13]. All cell lines were grown in plastic culture flasks (VWR, Canada) incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were subcultured at 72-h intervals using 0.25% trypsin-0.02% EDTA and seeded into fresh medium at a density of 2.5–3.5 × 10^5 cells/mL.

2.4. Western blot analysis

Cells were seeded in six-well plates at a density of 2 × 10^5 cells/well and cultured for 48 h. The cells were then lysed with cell lysis buffer (RIPA buffer, 100 μL) and quantified using a BCA array. Equal amounts of quantified total protein (20 μg, 10%) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were transferred to membranes and incubated with blocking buffer for 1 h. The blocked membrane was probed with an antibody against the laminin 332 γ2 chain (1:1000; clone D4B5; cat. no. MAB19562; Merck Millipore, Darmstadt, Germany) overnight at 4 °C. The blot was probed for glyceraldehyde phosphate dehydrogenase (GAPDH) as an internal control with anti-GAPDH antibodies (1:200; cat. no. sc-32233; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The blot was washed three times with TBS-T (0.1%) for 10 min each. The immunoreactive bands were detected by incubation with secondary antibodies (1:5000; anti-mouse IgG) for 1 h and then washed three times with TBS-T (0.1%) for 10 min each.

2.5. Real-time reverse transcription polymerase chain reaction (qRT-PCR)

For gene expression analysis, 2 × 10^5 cells were cultured for 48 h on different days (n = 3). Cells were washed with PBS, and

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