Available online at www.sciencedirect.com
ScienceDirect
Annals of Diagnostic Pathology 15 (2011) 46 – 51
Annals of DIAGNOSTIC PATHOLOGY

Cytologic-Pathologic Correlation

Nuclear and cytoplasmic Id-1 expression patterns play different roles in angiogenesis and lymphangiogenesis in gastric carcinoma

Chun-Feng Si[a], Jian-Qiang Guo[a], Yong-Mei Yang[b], Nan Zhang[a], Cheng-Ran Pan[a], Qing-hui Zhang[c], Ting-guo Zhang[c], Cheng-jun Zhou[a,*]

[a]*Department of Digestive Disease, The Second Hospital, Shandong University, Jinan, Shandong, 250033 PR China*
[b]*The Central Laboratory, Qi Lu Hospital, Shandong University, Shandong, PR China*
[c]*Department of Pathology, Qi Lu Hospital, Shandong University, Shandong, PR China*

Abstract The purpose of the study was to investigate the expression and impact of Id-1 (inhibitor of differentiation) on tumor progression, angiogenesis, and lymphangiogenesis in gastric adenocarcinoma. The study included 97 cases of gastric adenocarcinoma, which were surgically excised at the Second Hospital of Shandong University. Immunohistochemistry was used to detect the Id-1 expression, and dual-labeling immunohistochemistry was used to evaluate the microvessel density (MVD) and lymphatic vessel density (LVD). The Id-1 protein was mainly expressed with nuclear staining in well-differentiated carcinoma, but with cytoplasmic staining in moderately and poorly differentiated carcinoma, which showed a significant difference ($P < .0001$). Moreover, the expression patterns had different and crucial effects on angiogenesis and lymphangiogenesis. Nuclear staining of Id-1 inhibited angiogenesis, but cytoplasmic staining promoted angiogenesis (MVD, 110.57 ± 32.32 vs 141.45 ± 55.60) ($P < .05$). Consistent with their roles in angiogenesis, the nuclear and cytoplasmic expressions of Id-1 had similar effects on lymphangiogenesis: nuclear expression inhibited and cytoplasmic expression promoted lymphangiogenesis (LVD, 2.62 ± 1.03 vs 4.05 ± 2.04) ($P < .05$). Microvessel density and LVD showed no significant difference in low–and high–Id-1 expression groups ($P > .05$). Aberrant expression of Id-1 from nuclear to cytoplasm is accompanied with tumor malignant progression, which promotes angiogenesis and lymphangiogenesis; and Id-1 should be developed as a target for gastric carcinoma therapy.

Keywords: Id-1; LYVE-1; CD31; Angiogenesis; Lymphangiogenesis

1. Introduction

Id-1 (inhibitor of differentiation/DNA synthesis) protein is a member of the family of helix-loop-helix proteins. It lacks the basic domain to bind DNA and functions mainly as a dominant inhibitor of the bHLH transcription factor through heterodimerization [1]. Id-1 plays a critical role in regulating cell proliferation and differentiation [2,3]. The ability of Id proteins to inhibit differentiation may play an important role in tumorigenesis, as loss of differentiation is a hallmark of cancer cells. Id family members are highly expressed in neuroblastoma, prevent pharmacologic differentiation of neuroblastoma cells lines, and may indicate a poor prognosis [4-6]. Id-1 has been suggested to be involved in the malignant progression of human cancer, and Id-1 is constitutive in the highly aggressive cancer cells in breast cancer [7]. Recent studies have implicated a regulatory role of Id proteins in multiple processes such as cell cycle progression [8] and angiogenesis [9].

Our study suggests that aberrant expression of Id-1 from nuclear to cytoplasm should be accompanied with tumor malignant progression and that the roles in angiogenesis and lymphangiogenesis have been transformed.

* Corresponding author.
E-mail address: medicine@sdu.edu.cn (C. Zhou).

1092-9134/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.anndiagpath.2010.08.002

2. Materials and methods

2.1. Patients

The study included 97 patients with gastric adenocarcinoma who underwent primary surgical resection between 2007 and 2008 at the Second Hospital of Shandong University. The clinicopathologic information, including sex and age, was obtained from the clinical records. All the cases with gastric carcinomas were classified as well differentiated (n = 21), moderately differentiated (n = 34), poorly differentiated (n = 42) (Table 1). All the diagnoses were made following the *Pathology and Genetics of Tumours of the Digestive System* [10] by 3 pathologists.

2.2. Immunohistochemistry staining

Immunohistochemistry (IHC) was performed on 4-μm-thick routinely processed paraffin sections in series. Id-1 was observed with a rabbit polyclonal anti–Id-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA; C-20, dilution 1:400). Sections were dewaxed, and endogenous peroxidase was blocked by immersing the slides in a 3% solution of hydrogen peroxide in methanol for 10 minutes. This was followed by a step of antigen retrieval; the slides were immersed in 0.01 mol/L citrate buffer solution (pH 6.0) and placed in a microwave oven for 25 minutes. Following a wash in 1 mol/L phosphate-buffered saline (PBS, pH 7.4), the sections were covered with normal serum in a humidity chamber for 30 minutes at room temperature. Excess serum was rinsed off with 1 mol/L PBS, and the sections were incubated with the primary antibody in a humidity chamber for 45 minutes at room temperature. Afterward, the detection kit (Dako REAL Envision Detection System, Code K5007; Dako, Carpinteria, CA) was used; and the sections were incubated for 30 minutes at room temperature, washed in 1 mol/L PBS, and covered with 3,3′-diaminobenzidine tetrahydrochloride solution for 10 minutes under microscope according to the manufacturer's instruction. Sections were then immersed in running tap water and counterstained with hematoxylin for 1 minute, followed by tap water bath and immersion in a series of alcohol baths of increasing concentrations and xylene, and then covered with coverslips. Negative controls were performed, in which the primary antibody was omitted.

Table 1
The Id-1 expression and its relationship with differentiated grading in gastric carcinoma

Variables	No. of patients	Id-1 expression		Staining pattern of Id-1	
		Low	High	N	C
Patient no.	97	15	82	27	70
Sex					
Male	59	9	50		
Female	38	6	32		
Age, y					
≤59	32	5	27		
≥60	65	10	55		
Differentiated grading					
Well differentiated	21	3	18†	15*	6
Moderately differentiated	34	2	32†	4	30*
Poorly differentiated	42	10	32†	8	34*

N indicates nuclear; C, cytoplasmic.
* Significant: $P = .000 < .0001$.
† $P = .098 > .05$.

2.3. Double-labeling IHC staining of LYVE-1 and CD31

Microvessel density (MVD) and lymphatic vessel density (LVD) were detected with a mouse monoclonal anti-CD31 antibody (Abcam; 2F7; dilution, 1:700; Cambridge, CA) and with a rabbit polyclonal anti-LYVE1 antibody (Abcam; ab36993; dilution, 5 μg/mL). Simultaneous detection of blood and lymphatic vessels was performed in accordance with manufacturer's instructions (Histostain-DS Broad Spectrum; Zymed, South San Francisco, CA). Preparation of paraffin sections was done as described above; 0.05% Tween-20 PBS (pH 7.4) was used as the buffer. Staining was performed consecutively with LYVE1 as the first staining. LYVE1 and CD31 labeling was performed by different chromogens (5-bromo-4-chloro-3-inodlyl-phosphate (BCIP)/nitro blue tetrazolium (NBT) and 3-amino-9-ethyl-carbazole (AEC), respectively). Negative controls were included with omission of primary antibodies.

2.4. Evaluation of Id-1 staining

Histologic and IHC evaluations were performed by 3 pathologists independently. Slides with debated evaluation were reevaluated, and a consensus was reached. For each sample, at least 3000 carcinoma cells were evaluated for Id-1 expression. We examined the sections at 200× magnification (20× objective and 10× ocular), and the percentage of carcinoma cells with nuclear or cytoplasmic staining was determined. In addition, according to the percentage of stained tumor cells, Id-1 expression was classified as "low" (<66% positive cells) expression and "high" (≥66% positive cells) expression [11].

2.5. Evaluation of MVD and LVD in Id-1–positive areas

Microvessel density and LVD were assessed based on staining of CD31 and LYVE-1. Five areas of maximal MVD were identified by screening (magnification 40×). The number of vessels was counted within a counting grid at 200× magnification (20× objective and 10× ocular). For the vessels counts, any stained endothelial cell or cell cluster separated from another microvessel structure was considered as a countable microvessel. But a lumen was not necessary for a structure to be counted as a microvessel. LYVE-1–positive endothelial cells were blue staining (BCIP/NBT), and CD31-positive endothelial cells were red staining (AEC). The number of vessels was expressed as the mean value of counted microvessel in 5 evaluated grids in areas of maximum vessel density. Data were shown as the mean ± SD (Table 2).