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Cancer Detection and Prevention 30 (2006) 168–173

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Differential caspase-3 expression in noncancerous, premalignant and cancer tissues of stomach and its clinical implication

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Accepted 28 February 2006

Abstract

Background: Caspase-3 is a critical apoptosis-promoting element but its status during stepwise gastrocarcinogenesis needs to be further clarified. *Materials and methods*: By the use of frozen tissue microarrays constructed with the tissue spots cored from defined histological regions in tissue blocks, the pattern of caspase-3 expression in noncancerous, premalignant (atrophic gastritis and intestinal metaplasia) tissue and cancer spots were analyzed under the same experimental conditions by the methods of immunohistochemistry and mRNA-in situ hybridization. *Results:* Caspase-3 was expressed in all 34 of the noncancerous mucosa (100%), in 16 of the 17 premalignant tissues (94.1%) and in 15 of the 48 gastric cancers (31.3%). The incidences of caspase-3 detection were significantly different ($p < 0.01$) between noncancerous mucosa and intestinal as well as diffuse gastric cancers. *Conclusion:* Down-regulated caspase-3 is closely correlated with gastric cancer formation and would be a potential indicator of tumor formation and progression. *Helicobacter pylori* (*H. pylori*; Hp) infection is but not the only one element responsible to the enhanced caspase-3 expression in gastric epithelia.

Keywords: Gastric cancer; Frozen tissue microarray; Gene expression; Caspase-3; Apoptosis-promoting; Noncancerous epithelia; Premalignant; Immunohistochemistry; RNA isolation; mRNA in situ hybridization; Intestinal-type gastric cancer; Diffuse-type gastric cancer; Diagnosis; Prognosis

1. Introduction

Gastric cancer is one of the commonest malignant tumors in China as well as in the world. Long-term and stepwise progression is required for gastric cancer formation [1,2]. In accompany with the sequential histological changes during the oncogenic processes, the intrinsic regulatory systems for normal cell maintenance and death are disordered, leading the growth-limited gastric epithelial cells to an immortalized status with altered morphology and finally malignant phenotypes [3–5]. Therefore, gastric cancers have been recognized as a sort of apoptosis-deregulated disease [6].

Several molecular pathways can commit cells to apoptotic death, among which Fas/FasL/Caspases interaction is one of the important elements [7,8]. Accumulating evidences demonstrated that Fas receptor and its ligand FasL could lead many types of cancer cells to death via Caspase-mediated cascade [9,10]. Soluble Fas and Fas ligand were found frequently in gastric cancers and their related lesions [11–13] but the corresponding data concerning caspases' family, a group of downstream effectors of Fas-mediated apoptotic signaling pathway, have not yet been well documented [14,15]. Caspase-3 is the core member of caspase family, because of its pivotal roles in connecting upstream death signals and downstream apoptotic substrates, such as poly-(ADP-ribose) polymerase (PARP), DNA fragmentation factor and protein kinase C (PKC) [16–18]. Consequently, DNA molecules are cut into fragments by activated endonuclease [18]. Recent cDNA microarray data revealed that the level of caspase-3 expression was

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0361-090X/$30.00
doi:10.1016/j.cdp.2006.02.004

lower in the gastric tissues obtained from gastric cancer patients in comparison with that of normal gastric mucosa [19]. However, the fidelity of those data and its correlation with stepwise gastrocarcinogenesis remains to be further validated. Caspase-3 expression in different gastric tissues was therefore characterized using high throughput frozen tissue microarrays.

2. Materials and methods

2.1. Surgical specimens

Ninety-nine gastric tissue specimens from 48 GC cases were selected from the Frozen Gastric Tissue Bank of Cancer Institute, Dalian Medical University (DMU). Among them, 11 cases were diagnosed with and 9 cases without *Helicobacter pylori* (*H. pylori*; Hp) infection. After getting patients' consents, the specimens were collected from the operation rooms of DMU affiliated hospitals. Briefly, the tissues were chosen and incised carefully from the tumor mass, tumor-surrounding tissue and grossly normal-looking epithelium, respectively. They were trimmed into suitable size on ice, snap-frozen immediately in liquid nitrogen and stored at −85 °C until use. All treatments were done within 20 min after removal. Detailed patients' medical records were collected as well.

The frozen tissue blocks were sectioned in 7 μm thickness, fixed in cold acetone for 20 min and subject to hematoxylin and eosin (H & E) staining for pathological and cell composition evaluation. If only one cell type exists in the tissue block, further sections were made for direct RNA and protein preparations and tissue array construction; otherwise, the target histological regions were marked for tissue-defined RNA and protein preparations followed by tissue array construction.

2.2. Tissue defined RNA isolation and frozen tissue array construction

The target region(s) in the slides was determined by conventional pathological observation and its margin was marked in a series of inked spots with a special instrument named Lencil (Chinese Inventive Patent: 02109826.3), a lens-like device fixed on the light microscope with a spray tip that could mark the central part of the visual field exactly when upward pressure was formed by attaching the slide with the spray tip. Referring to the marks on the section, the corresponding region in the frozen tissue block was segregated with an autoclaved knife in the depth of 0.1–0.2 mm. The block was then subjected to serial frozen section. The target fragment was separated from the main section and collected by a tip wetted with Trisol (Life Technology, USA). According to the size of the interested region, four to eight pieces of 7 μm serial sections were collected for RNA isolation by the method described elsewhere [20].

Frozen tissue arrays were constructed on the same regions used for RNA preparation by the methods described recently [21]. The special core biopsy needle in 0.6 mm inner diameter was punched into the target region and the collected tissue was filled into the pore immediately. The case orders in the array block were recorded and all performances were done under −30 °C.

2.3. Immunohistochemical (IHC) staining

The frozen GC tissue arrays in the density of 36 spots/0.5 cm^2 or 42 spots/0.7 cm^2 were constructed with the tissues cored from selected histological regions. The frozen sections in 7 μm thickness were used for immunohistochemical evaluation of the expression level and intracellular distribution of caspase-3 in different gastric tissues under the same experimental conditions [21]. Rabbit anti-human caspase-3 antibody (Santa Cruz, CA, USA) and a two-step polymer detection reagent (Zymed Inc., USA) was used in immunohistochemical staining by the methods described elsewhere [22]. The array sections without primary antibody incubation were used as background controls. Based on the background-subtracted labeling density in parenchyma cells of individual spots, three researchers in our group evaluated the staining results separately and their judgments were summarized in the form of negative (−), weakly positive (+), moderately positive (++) and strong positive (+++).

2.4. mRNA in situ hybridization analyses (RISH)

Frozen tissue arrays were sliced in 7 μm thickness. The sections were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for RISH. The paraformaldehyde-fixed slides were rinsed with PBS three times for 5 min each, emerged in 0.5% H_2O_2 containing formaldehyde solution at 37 °C for 30 min and incubated with 3% citrate acid-diluted digestive solution. The sections were then covered with 50 μl hybridization solution containing 0.5 μg of digoxigenin-labeled human caspase-3 RNA probe (Haoyang, Co. Ltd., Tianjin, PR China) at 37 °C for 90 min, followed by incubating with stabilization solution and blocking solution for 20 min, respectively, with rabbit anti-digoxigenin antibody for 60 min and with biotinylated anti-rabbit IgG and streptavidin–biotin complex for 20 min. The hybridization signal was visualized with 3′-diaminobenzidine tetrahydrochloride. The section incubated with hybridization buffer without probe supplementation was used as negative and background control.

2.5. Statistical analysis

The Kruskal–Wallis test and Mann–Whitney test were utilized to compare: (i) the distributions of caspase-3