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Cancer Letters 223 (2005) 339–347
CANCER Letters
www.elsevier.com/locate/canlet

Correlation of Wnt-2 expression and β-catenin intracellular accumulation in Chinese gastric cancers: relevance with tumour dissemination

Xiao-Xin Cheng[a], Zi-Chuang Wang[a], Xiao-Yan Chen[a,b], Yuan Sun[b], Qing-You Kong[b], Jia Liu[a,b], Hong Li[a,b,*]

[a]*Cancer Institute, College of Basic Medical Sciences, Dalian Medical University, Dalian 116027, People's Republic of China*
[b]*Laboratory of Cell Biology and Molecular Genetics, College of Basic Medical Sciences, Dalian Medical University, Dalian 116027, People's Republic of China*

Received 21 September 2004; received in revised form 30 October 2004; accepted 2 November 2004

Abstract

Wnt/β-catenin signalling pathway is integrally associated with human tumour development and progression. Aberrant β-catenin intracellular distribution has been found in gastric cancer, but the pattern of Wnt expression in stepwise gastrocarcinogenesis and its potential influence in β-catenin distribution are still lesser known. By the methods of frozen tissue array-based immunohistochemistry, Western blot analysis and RT-PCR, a paralleled study was conducted to check Wnt2 expression and β-catenin intracellular distribution in two major subtypes of gastric cancers (intestinal gastric cancer, i-GC and diffuse gastric cancer, d-GC) and their premalignant (intestinal metaplasia, IM and chronic gastritis, CG) and noncancerous counterparts. According to the results obtained and the clinical data collected, correlation of Wnt2 expression with β-catenin translocalisation and their links with tumour dissemination were elucidated. The results demonstrated (1) that Wnt2 expression and cytoplasmic/nuclear β-catenin accumulations appeared in most gastric cancers irrespective to their morphological phenotypes, (2) that over-expressed Wnt and nuclear translocalisation of β-catenin were found in 68 and 58% of i-GCs and in 47 and 47% of d-GCs in a closely related pattern ($P<0.01$) and (3) that co-existence of Wnt2 up-regulation/β-catenin nuclear translocalisation were positively associated with lymph node metastasis ($P<0.05$) as well as T-stage. These data indicate that Wnt/β-catenin signalling pathway is activated in most of gastric cancers, which may play pivotal roles either in gastric cancer formation or in tumour invasion and dissemination.

Keywords: Wnt2; β-catenin; Gastric cancer; Tumour progression; Lymph node metastasis

1. Introduction

Gastric cancer (GC) is one of the commonest malignancies in China and some parts of the world. Although the approaches for earlier detection,

* Corresponding author. Address: Cancer Institute, College of Basic Medical Sciences, Dalian Medical University, Dalian 116027, People's Republic of China. Tel./fax: +86 411 472 1202.
E-mail address: lihongmcn@yahoo.com.cn (H. Li).

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doi:10.1016/j.canlet.2004.11.013

diagnosis and treatments have been somewhat improved, the prognosis of this disease remains still poor due to the strong tendency of local invasiveness and distal metastasis of the GC cells [1,2]. Therefore, exploration of underlying molecular mechanism of GC dissemination would be of clinical significance.

Invasion and metastasis are complex processes that require multiple genetic alterations and, more importantly, their coordinated interactions. Among the metastasis-associated factors, the roles of Wnt genes have been drawn researchers' attention because of their tumour promoting effects through altering intracellular distribution of β-catenin [3,4]. The translocalised β-catenin can activate T-cell factors (TCFs) that in turn enhance the transcription of multiple target genes including some metastasis-associated genes such as CD44 [5] and MMP-7 [6].

Accumulating evidences have demonstrated that translocalised β-catenin is commonly observed in both intestinal and diffuse gastric cancers, indicating its involvement in gastric cancer formation [7]. cDNA microarray-based gene expression profiling showed that the expression levels of Wnt2 and Wnt5a are up-regulated in GCs regardless of their histological phenotypes [8]. Similar phenomena were also observed with other experimental approaches [9–11]. However, more focused studies on the correlation of Wnt2 expression and the altered β-catenin distribution in GCs are still limited. To address this issue, a comprehensive investigation was conducted here using the RNA and protein samples isolated from defined histological regions of gastric tissue blocks and the frozen tissue microarrays constructed with the tissues cored from the same regions.

2. Materials and methods

2.1. Surgical specimens

The surgical specimens of 38 GC cases were selected from The Frozen Gastric Tissue Bank of Cancer Institute, Dalian Medical University (DMU). After getting patients' consents, the specimens were collected from the operation rooms of DMU affiliated hospitals. The tissues were chosen and incised carefully from tumour mass, tumour-surrounding tissue and grossly normal-looking epithelium, respectively. They were trimmed into suitable sizes on ice, snap-frozen immediately in liquid nitrogen and stored at −85 °C until use. All treatments were done within 20 min after removal.

Before further experiments, the pathological experts in Cancer Institute of DMU performed H&E staining and histological examination on each of the selected tissue blocks. When the blocks showed homogeneous composition, they were sectioned directly for RNA and protein isolation following by tissue array construction. Otherwise, the target histological region(s) was defined and sampled for RNA/protein isolations and tissue array construction by the methods described elsewhere [12]. Altogether, 67 representative histological regions were chosen in this study.

2.2. Immunohistochemical 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 and sectioned in 7 μm thickness and used for immunohistochemical profiling of Wnt expression and the patterns of β-catenin distribution under the same experimental conditions. By the method described previously [13], immunohistochemical staining was performed using goat anti-human Wnt2 polyclonal antibody (1:150) and mouse anti-human β-catenin monoclonal antibody (1:200) (Santa Cruz, CA, USA), respectively. The array sections without primary antibody incubation were used as background controls. Based on the labelling density, two independent researchers evaluated the staining results and scored them as negative (−), weakly positive (+), moderately positive (++) and strong positive (+++).

2.3. RT-PCR for Wnt2

RT-PCR was performed using Takara RNA PCR kit (AMV) version 2.1(Takara Dalian Inc., Dalian, China) under the following conditions: 0.8 μg total RNA was reverse-transcribed with random primer at 55 °C for 30 min in a 20 μl solution; the reaction was terminated by incubating the mixture at 99 °C for 5 min. Wnt2 gene was amplified using a pair of