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Original article

ING1a and ING1b different expressed in sporadic hepatocellular carcinoma

Différente expression de l'ING1a et l'ING1b dans le carcinome hépatocellulaire sporadique

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Abstract

Background. – Inhibitor of Growth 1 (ING1) is recognized as candidate tumor suppressor. The expression of ING1 in human malignances is discordant, having a tissue dependant manner. ING1a and ING1b are the major isoforms of ING1 in most of human tissues. It has been proved that these two isoforms had different functions. The respective expressions of ING1a and ING1b in hepatocellular carcinoma are remained to investigate.
Methods. – The general expression level of ING1 and the transcription levels of ING1a and ING1b in 31 pairs of hepatocellular carcinoma and matched nontumorous tissues were evaluated by using immunostaining and semi-quantitative reverse transcript polymerase chain reaction.
Results. – ING1 was expressed in all tissues, and was mainly localized in the nuclei of hepatocytes or hepatoma cells. ING1b was up-regulated in the HCCs with advanced clinic stages or poorly differentiated grades, compared with the matched tissues ($P < 0.01$). ING1a expression level had no obviously enhancement.
Conclusions. – ING1b was up-regulated in HCC during the progression process and might contribute the alternation of general expression level of ING1.

Résumé

Objectifs. – L'inhibiteur de croissance (*inhibitor of growth 1*, [ING1]) est connu comme un candidat de suppresseur tumoral. L'expression de l'ING1 dans les maladies malignes humaines est discordante et dépendante de différents tissus. L'ING1a et l'ING1b sont les majeurs isoformes de l'ING1 dans la plupart de tissus humains et chacun a ses fonctions différentes. L'expression de l'ING1a et l'ING1b dans le carcinome hépatocellulaire sont restés à étudier.
Méthodes. – L'expression niveau de l'ING1 et le transcriptionnel niveau de l'ING1a et l'ING1b dans 31 paires de carcinome hépatocellulaire et les tissus autour de tumeur sont évalués par l'immunohistochimie et le semi-quantitative *reverse transcript polymerase chain reaction.*
Résultats. – L'ING1 était exprimé dans tous les tissus et se situait dans le noyau de cellules. Niveau d'expression de l'ING1 et de transcription de l'ING1b étaient sus-régularisés dans le HCC, notamment dans les stades avancés clinique ou les grades peu différentiels, par rapport aux ceux dans les tissus non tumoraux($p < 0{,}01$). Au contraire, le niveau d'expression de l'ING1a n'avait pas de changement statistique.
Conclusions. – La sus-régularisée expression d'ING1b dans le HCC avancé ou peu différentielle peut être causer l'alternation d'expression de l'ING1.

Keywords: ING; ING1b; HCC; Isoforms; RT–PCR; Immunohistochemical

Mots clés : ING ; ING1b ; HCC ; Isoforms ; RT–PCR ; Immunohistochimie

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1. Introduction

A candidate tumor suppressor, inhibitor of growth 1 (ING1), was cloned by Garkavtsev et al. in 1996 [1]. It was shown to be localized on chromosome 13q34 [2], and comprised of four exons (1a, 1b, 1c and 2). ING1 encodes four isoforms, $p47^{ING1a}$, $p33^{ING1b}$, $p24^{ING1c}$ and $p27^{ING1d}$, due to alternative splicing of its mRNA product and usage of different promoter regions. Each isoform of ING1 has a distinct *N*-terminus, but contains an identical *C*-terminus, encoded by exon 2, that harbors a nuclear localization signal (NLS), containing two distinct nucleolar translocation sequences (NTS), and a plant homeodomain (PHD) form of C4HC3-type zinc finger spanning 50–80 amino acid residues [3]. The primary structures of ING1 isoforms imply that different isoforms of ING1 have different functions. $p47^{ING1a}$ and $p33^{ING1b}$ are the major isoforms of human ING1 [4], they had been found having different effects on chromatin remodeling [5], inducing or enhancing apoptosis [6] and gene expression regulation [7] in the past 10 years.

On one hand, most of studies supported that decreased expression of ING1 was an important event in some human cancers, including breast carcinomas [8], leukemia [9], gastric carcinomas [10] and squamous cell carcinomas [11]. On another hand, several studied revealed that expression of ING1 was increased in melanoma cell lines [12], basal cell carcinomas [13], gliomas [14,15] and transitional cell carcinomas [16]. The respective expressions of ING1a and ING1b in hepatocellular carcinoma are remained to investigate.

In this paper, we investigated whether ING1a and ING1b presented different transcriptional patterns in sporadic hepatocellular carcinoma (HCC).

2. Materials and methods

2.1. Tumor and matched nontumorous tissues

Thirty-one pairs of tumor and matched nontumorous tissues were obtained from patients with HCC who underwent liver transplantation in Orient Organ transplant center, Tianjin, China from January 2005 to June 2005. Informed consent was obtained from each patient prior to tissue acquisition. For RNA preparation, the resected tissues were flash frozen immediately (within 5 min) upon removal and stored at −80°C. The remaining tissues were used for routine histopathological examination. All the slides were examined microscopically by two pathologists independently. Tumors were classified according to the grades of primary liver carcinoma as defined by Edmondson and Steiner [17], grade 1 ($n = 1$), grade 2 ($n = 10$), grade 3 ($n = 18$), grade 4 ($n = 2$). All the patients were classified according the criteria of American Joint Committee on Cancer (AJCC) TNM staging manual [18], stage I ($n = 2$), stage II ($n = 9$), stage IIIA ($n = 8$), stage IIIB ($n = 12$), stage IIIC ($n = 0$), stage IV ($n = 0$).

2.2. Immunostaining

Sections (6-μm) cut from paraffin-embedded blocks were dewaxed by passage through xylene and then rehydrated in graded alcohol (100, 90, 80 and 70%). Endogenous peroxidase activity was blocked by incubating the section in 3% hydrogen peroxide solution for 10 min. Slides were then pretreated in a Tris–EDTA solution (pH9.0) for 20 min at 98 °C to unmask tissue antigen. Endogenous peroxidase activity was quenched in a 0.3% for 10 min. After rinsing with 0.01 mol/l phosphate-buffered saline (PBS), pH 7.4, nonspecific antibody binding was reduced by incubating the sections with 2% horse serum for 40 min. After decanting excess serum, sections were incubated overnight at 4-°C with a mouse antihuman $p33^{ING1b}$ monoclonal antibody (BD biosciences, CA, USA) at a 1:400 dilution in PBS. The sections were developed with PV-9000 polymer detection system (Golden Bridge International, USA) and diaminobenzidine tetrahydrochloride (DAB). All slides were counterstained with hematoxylin after immunostaining.

Five random images of each slide were taken by Leica DMIL microscope and Leica DC-300F digital camera under the same exposure factors at a magnification of 400×, and saved as TIFF format at a resolution of 1,390 × 1,040. Motic Med 6.0 software (Motic China Group CO., LTD, XiaMen, China) was employed to select positive stain nuclei and calculate the integrated optical density (IOD) value of each image. All images were evaluated by a same macro.

2.3. Semiquantitative reverse transcript polymerase chain reaction (RT–PCR)

Total RNA was isolated using Trizol reagent (Invitrogen Corporation, Peking, China) at first. Then, synthesis of cDNA was done with 2 μg total RNA using first-strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Inc., NJ, USA). cDNA samples were store at −20 °C until PCR reactions were performed. At last, PCR was performed in 25 μl reaction mixtures comprising 2 μl cDNA, 2.5 μl 10 × buffer, 1 μl 2.5 mol/l deoxynucleotide triphosphate mixture (dNTP mixture), 2 U Taq DNA polymerase (TaKaRa, Dalian, China), 0.5 μl of 10 mmol/l each primer of ING1a or ING1b or GAPDH, and adjusted the reaction system to 25 μl by sterilized ddH_2O. Intron-spanning primers were: for ING1a, 5′-GGCTCGGAGA-CAGTTTCAGG-3′ and 5′-CCACCATCTGGC- TCACG-3′ (376 bp); and for ING1b, 5′-TGGAGGAAGCGGAAAGC-3′ and 5′-CTTGCTG- TTGGGCTTGTC-3′ (495 bp). Primers for GAPDH were 5′-ACCACAGTCCATGCCATC- AC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′ (432 bp). ING1a and ING1b were amplified through 32 cycles, each amplification consisting of denaturation at 94 °C for 1 min, primer annealing at 58 °C for 30 s and extension at 72 °C for 40 s. Cycles were preceded by incubation at 94 °C for 5 min to ensure full denaturation of DNA strands, and followed by an extra incubation at 72 °C for 10 min to ensure full extension of the products. GAPDH were amplified through 26 cycles. Products of RT–PCR were electrophoresed on 15 g/l agarose gel. The light intensities of DNA bands were scanned by Gel documentation systems (UVItec Limited, Cambridge, UK). Reproducibility was confirmed by processing all samples at least twice.