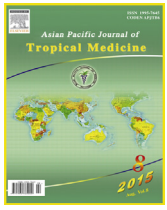
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2015.07.007

Correlation of survivin, p53 and Ki-67 in laryngeal cancer Hep-2 cell proliferation and invasion

Shi-Geng Pei[1*], Ju-Xiang Wang[2], Xue-Ling Wang[1], Qing-Jun Zhang[1], Hong Zhang[1]

[1]*Department of Otolaryngology Head and Neck Surgery, Affiliated Hospital of Hebei Engineering University, 056002, Handan, China*

[2]*Hebei Engineering University Medical College, 056002, Handan, China*

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 20 Jun 2015
Accepted 15 Jul 2015
Available online 22 July 2015

Keywords:
Survivin
p53
Ki67
Hep-2
Transwell
Laryngeal cancer

ABSTRACT

Objective: To investigate the mechanism of survivin, p53 and Ki-67 on Hep-2 human laryngeal cancer endothelial cell proliferation and invasion.
Methods: Laryngeal squamous cell carcinoma and paracancerous normal tissues were collected, total RNA was extracted from tissues, *survivin*, *p53* and *Ki-67* gene mRNA expression levels in laryngeal cancer and the adjacent tissues were detected by Real-time PCR. Human laryngeal cancer Hep-2 epithelial cells were selected, *survivin* gene was overexpressed, and cell proliferation was detected by MTT. *p53* and *Ki-67* gene expression changes in overexpressed *survivin* gene were detected by Western blot. Changes in Hep-2 cell invasive ability were studied when *survivin* was overexpressed as detected by Transwell invasion assay.
Results: In the adjacent tissues, *survivin*, *p53* and *Ki-67* gene relative expression levels were 1.72 ± 0.9, 13.7 ± 5.7 and 5.7 ± 1.3, respectively; while in cancer tissues, gene relative expression levels were 53.7 ± 8.3, 66.7 ± 5.2 and 61.0 ± 3.1, respectively, which was significantly increased. As detected by MTT, relative cell survival rate within 12 h of *survivin* overexpression were: load control group (88.5 ± 1.6)%; overexpressed group (90.3 ± 1.9)%. Transwell invasion assay results indicated that overexpressed *survivin* could significantly increase the relative survival rate of cells.
Conclusions: Expressions of *p53*, *Ki67* and *survivin* are increased in cancer; and there is a positive correlation between *survivin*, *p53* and *Ki67* expressions in laryngeal carcinoma.

1. Introduction

Laryngeal cancer is a common malignancy that is secondly high estin disease incidence only after nasopharyngeal cancer in otolaryngology. Although the occurrence of cancer is a multifactorially complex process, it is generally believed that in cancer gene activation, tumor suppressor gene inactivation and overexpression of anti-apoptotic genes are important causes of cancer [1–3].

In recent years, *survivin* has been found to be an apoptosis inhibiting gene. It is a member of the apoptosis inhibitory protein factor family, which is tumor specific and only expressed in tumor and embryonic tissues; because it is expressed in a variety of tumors and highly correlated with tumor diffusion transfers. Therefore, it has become a hot topic in cancer research. Studies have found that survivin upregulation and p53 overexpression are closely related to nasal type NK/T-cell lymphoma. Survivin might prompt p53 to coordinate cell cycle regulation. Ki67 is a kind proliferation associated with nuclear antigens. In addition, it is expressed to all G_0 phase cell cycles, and closely related with tumor differentiation, invasion and metastasis [4–8]. Therefore, in this study, we discuss survivin with p53 and Ki-67 expressions in laryngeal carcinoma and its correlation with Hep-2 cell proliferation and invasiveness in tissue and cell levels; in order to explore the role of survivin in tumor occurrence and development.

2. Materials and methods

2.1. Materials

2.1.1. Hep-2 cell line

Hep-2 cell line was purchased from ATCC, and stored in liquid nitrogen out of laboratory. Cells were cultured in DMEM

*Corresponding author: Shi-Geng Pei, Professor, Department of Otolaryngology Head and Neck Surgery, Affiliated Hospital of Hebei Engineering University, No. 81, Congtai Road, 056002, Handan, Hebei Province, China.
Tel: +86 18031028060
E-mail: psg334@126.com

Peer review under responsibility of Hainan Medical University.
Foundation Project: It is supported by Handan Science and Technology Bureau (No. 1323108088).

complete medium supplemented with 10% fetal bovine serum (Lanzhou MinHai Bio-engineering Co., Ltd) at 37 °C with a 5% CO_2 environment.

2.1.2. Drug reagents and equipment

Tissue RNA extraction kit (RNeasy Plus Mini Kit) was purchased from QIAGEN; Reverse transcription kit (iScriptcDNA Synthesis Kit) was purchased from Bio-Rad; Real-time fluorescence quantitative PCR kit (SsoAdvanced SYBR Green Super mix) was purchased from Bio-Rad; Transfection Reagent lipofectamine 2000 was purchased from Invitrogen; MTT kit was purchased from Promega; Survivin monoclonal antibody was purchased from Abcam; p53 and Ki67 antibodies were purchased from Santa Cruz Biotechnology; Transwell chamber was purchased from Millipore; Matrigel matrix (5 mg/mL) was purchased from BD.

CO_2 incubator was from SANYO; UV spectrophotometer (SmartSpec 3000) was from Bio-Rad; Shimadzu (Precision Balances) was from Sartorius; Fluorescence quantitative PCR detection system (CFX96 Touch) was from Bio-Rad.

2.2. Methods

2.2.1. Clinical tissue samples

Surgical specimens from 64 cases of laryngeal squamous cell carcinoma and 34 cases of the adjacent normal tissues were collected from January 1997 to January 2009 at the Department of Pathology, Affiliated Hospital of Hebei Engineering University. Among the 64 cases of laryngeal cancer patients, 52 were male and 12 were female; meanwhile, 45 of them were ≤60-years-old and 19 were >60-years-old, with the average age of these patients being 57.6. Clinical staging was based on the Union for International Cancer Control 1997 standards; accordingly, 9 cases were stage-I, 20 cases were stage-II, 28 cases were stage-III, and 7 cases were stage-IV. Among these cases, 31 cases had cervical lymph node metastasis, while 33 cases were without cervical lymph node metastasis. After the follow-up for 3–10 years, there were 14 deaths and 50 survival patients in 3 years, and two lost cases and 46 survival patients in 5 years.

Mortar was added liquid nitrogen and tissues were grinded to powder. Tissue RNA extraction kit was used to obtain total RNA and preserved in a freezer at −80 °C.

2.2.2. Real-time PCR assay method

Clinical cancer and the adjacent normal tissue samples were collected, and separately randomized three cases into a single sample. *Survivin*, *p53* and *Ki67* gene mRNA expression levels were detected in tissue total RNA of all samples. According to reverse transcription kit instructions, tissue total RNA was reverse transcribed to cDNA, and related genes were detected by real-time PCR. *Survivin*, *p53* and *Ki67* gene mRNA sequences were queried from the NCBI database to design Real-time PCR primers. All primers were synthesized by SBS Genetech Co., Ltd. Specific sequences are as follows (Tables 1–3). Gene amplification Ct value was determined by Real-time PCR, and Ct values were negatively correlated to the initial DNA copy number. *GAPDH* was treated as internal control using the relative quantification method. For the homogenization process, each sample was analyzed by the ΔCt value; ΔCt = target gene Ct-reference gene Ct.

2.2.3. Plasmids construction and cell transfection

Survivin expression plasmid coding sequences obtained from the *survivin* gene were inserted between the two restriction sites, *Bam*H I and *Xho* I, in the pcDNA3.1 plasmid (added EGFP tag to detect the expression). Plasmid constructs were carried out by the Beijing ComWin Biotech Co., Ltd.

An appropriate number of cells were inoculated through culture plates, and 500 μL of complete medium was added into each well, causing transfection cell density to reach 70%–80%. Using 50 μL of serum-free medium, 1 μL of Lipofectamine 2000 was diluted. Then, the diluted plasmid and Lipofectamine 2000 were mixed, and incubated at room temperature for 20 min. After 4–6 h of culture, replace with fresh complete medium, and continued to culture cells.

2.2.4. Cell proliferation detected by MTT

In accordance with the manufacturer's instructions of the MTT Kit (Promega, G3582), a small amount of CellTiter 96® Aqueous One Solution Reagent was directly added into culture wells and incubated for 1–4 h, and absorbance was recorded on a 96-plate reader at 490 nm. At 490 nm, the measured absorbance values and the number of living cells in cultures was directly proportional.

2.2.5. Western-blotting

Cells were collected and lysed using 50 μL of RIPA lysis buffer, simultaneously added with a protease inhibitor cocktail (added 10 μL of cocktail to 1 mL RIPA by volume ratio), and mixed by pipetting. After placed on ice for 30 min, cells were sonicated, and ultrasound probes were used to deliver a short shock on ice at an appropriate frequency, cleavage cocktail at 4 °C, and centrifuged at 13000 r/min for 20 min. The

Table 1
Primers used in real-time PCR.

Gene	Accession no.	Primer (5′–3′)
BIRC5	NM_001012271.1	For: TGTCATAGAGCTGCAGGGTG Rev: GTCGAGGAAGCTTTCAGGTG
TP53	NM_001003210.1	For: TCAACAAGATGTTTTGCCAACTG Rev: ATGTGCTGTGACTGCTTGTAGATG
MKI67	NM_001145966.1	For: CCACACTGTGTCGTCGTTTG Rev: CCGTGCGCTTATCCATTCA
GAPDH	NM_002046	For: TGGAAGGACTCATGACCACA Rev: TTCAGCTCAGGGATGACCTT

BIRC5, baculoviral IAP repeat-containing 5; TP53, tumor protein p53; MKI67, antigen identified by monoclonal antibody Ki-67; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; For, forward; Rev, reverse.