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Original contribution

Aberrant expression of β-catenin and E-cadherin is correlated with poor prognosis of nasopharyngeal cancer

Lina Xu MD[a,1], Yi Jiang MD[a,1], Jun Zheng MD[a,b], Guiyuan Xie MD[c], Jiao Li MD[a], Lei Shi PhD[a], Songqing Fan PhD[a,*]

[a]*Department of Pathology, the Second Xiangya Hospital of Central South University, Changsha, 410011, Hunan, China*
[b]*Department of Biochemistry, Changzhi Medical College, Changzhi, Shanxi, 046000, China*
[c]*Department of Oncology, the Second Xiangya Hospital of Central South University, Changsha, 410011, Hunan, China*

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Summary Nasopharyngeal carcinoma has a high incidence in southern China. The Wnt/β-catenin signaling pathway plays a major role in cancer development and progression. Our current study aims to determine the clinical significance of the Wnt/β-catenin pathway components such as β-catenin, cyclooxygenase 2, cyclin D1, c-Myc, and E-cadherin in 148 nasopharyngeal carcinomas by immunohistochemistry. We found that nasopharyngeal carcinoma stage T_3+T_4 had significantly higher expression of β-catenin, cyclooxygenase 2, cyclin D1, and c-Myc and lower expression of E-cadherin than nasopharyngeal carcinoma stage T_1+T_2 ($P < .001$, $P < .05$, respectively).There was significantly higher expression of β-catenin ($P = .001$) and cyclooxygenase 2 ($P = .003$) and lower expression of E-cadherin ($P = .001$) in nasopharyngeal carcinoma with lymph node metastasis than in nasopharyngeal carcinoma without lymph node metastasis. The expression of β-catenin in nasopharyngeal carcinoma was positively correlated with cyclooxygenase 2 ($r = 0.458$, $P < .0001$), cyclin D1 ($r = 0.700$, $P < .0001$), and c-Myc expression ($r = 0.144$, $P = .006$) but negatively correlated with E-cadherin expression ($r = -0.601$, $P < .0001$), respectively. The univariate analysis confirmed that overexpression of β-catenin and cyclooxygenase 2 and decreased expression of E-cadherin were significantly correlated with disease-free survival ($P < .01$, $P < .05$, respectively). Overexpression of β-catenin and cyclooxygenase 2 and reduced expression of E-cadherin significantly correlated with a poor prognosis ($P = .005$, $P = .044$, $P = .019$, respectively) by Kaplan-Meier survival curves and the log-rank test. Multivariate analysis indicated that high expression of β-catenin and decreased expression of E-cadherin were independent prognostic factors ($P = .002$, $P = .011$, respectively) regardless of TNM stage and lymph node status. In conclusion, the aberrant high expression of β-catenin and decreased expression of E-cadherin is associated with poor prognosis in nasopharyngeal carcinoma.

1. Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common cancers in Southern China and Southeast Asia. It poses one of the serious health problems in southern China where an annual incidence of more than 20 cases per 100 000

* Corresponding author.
E-mail address: fansongqing2003@hotmail.com (S. Fan).
[1] Lina Xu and Yi Jiang contribute equally to this work.

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is reported [1]. The carcinogenesis of NPC is thought to be associated with a complex interaction of genetic, viral, environmental, and dietary factors. The molecular pathogenesis of NPC includes alteration of expression and function of multiple genes, including dominant oncogenes and recessive oncogenes or tumor-suppressor genes, and alterations in signaling pathways [1,2]. Further elucidation of the molecular mechanism underlying NPC is essential for the development of new effective therapeutic agents.

Aberrant activation of Wnt/β-catenin signaling is involved in the development of tumors. The β-catenin protein is a key mediator of canonical signaling in the Wnt/β-catenin signaling pathway [3,4]. Elevated β-catenin can translocate to the nucleus where it forms a complex with T-cell factor/lymphoid enhancer binding factor to activate the expression of a set of tumor-related target genes, such as cyclin D1, c-Myc, E-cadherin, cyclooxygenase 2 (COX-2), peroxisome proliferator–activated receptor δ, and matrix metalloproteinase 7, which, in turn, regulate cell proliferation and survival [3,4]. In particular, increasing evidence indicates that aberrant activation of the Wnt/β-catenin signaling pathway is found in a variety of human malignancies, including head and neck carcinoma, breast cancer, lung cancer, colorectal cancer, hepatocellular carcinoma, transitional cell carcinoma of the bladder, and melanoma [3-12]. The protein expression of several key signaling molecules in the Wnt/β-catenin pathway such as β-catenin, E-cadherin, c-Myc, cyclin D1, and COX-2 in NPC tissues had been studied [1,2,5,13,14,16-20,22-24]. However, as mentioned above, the associations between the abnormalities of these proteins and NPC status were mainly found by studies on a limited number of NPC cases by different research groups. Therefore, a study on a larger scale is needed to identify the roles these proteins play and the clinical significance that they have in NPC. Such a comprehensive study is difficult to carry out if it is done by immunohistochemistry (IHC) in conventional full-tissue sections from a large number of samples. In the present study, we take advantage of high-throughput NPC tissue microarrays (TMAs) to investigate the expression of β-catenin, COX-2, cyclin D1, c-Myc, and E-cadherin in NPC. Our present study should shed light on the relationship between expression of several key signaling molecules in the Wnt/β-catenin pathway and clinical features of NPC.

2. Materials and methods

2.1. TMAs and clinicopathologic data

In this study, we used the high-throughput NPC TMAs containing 148 NPCs that were constructed by the Cancer Research Institute, Xiangya School of Medicine, Central South University (Changsha, Hunan, China) and were kind gifts from Prof Guiyuan Li (Central South University, Hunan, China). All tumor samples are from primary NPC patients. No patients had been treated with radiotherapy or chemotherapy at the time of original biopsy. The detailed clinical characteristics of these patients were previously published [25,26]. Using the World Health Organization histologic classification of NPC and the TNM classification of malignant tumors, the NPC histologic patterns and clinical T stages were classified as follows: 127 cases of differentiated nonkeratinizing carcinomas (NKCs), 17 cases of undifferentiated carcinomas (UCs), and 4 cases of poorly differentiated keratinizing squamous cell carcinomas (KSCCs); 45 cases of stage T1, 50 cases of stage T2, 38 cases of stage T3, and 15 cases of stage T4. Among all patients included in the study, 100 patients had cervical lymph node metastasis (N1, 78; N2, 22), and 48 patients were cervical lymph node negative (N0, 48). Because no case revealed distant metastasis, all of the cases were classified as M0. Complete clinical record and follow-up data of all patients were available. Overall survival time was calculated from the date of diagnosis to the date of death or the date last known alive. A total of 72 patients (48.7%) were alive with a mean follow-up period of 78 months (10-125 months).

2.2. IHC and scoring

The IHC staining for samples on the TMAs was carried out using ready-to-use Envision + Dual Link System–HRP methods (Dako, Carpinteria, CA). The staining conditions for each antibody were adjusted according to previous data from the literature and our laboratory experience [14]. Briefly, each TMA section was deparaffinized and rehydrated, and high-temperature antigen retrieval was achieved for all antibodies by heating the samples in 0.01-M citrate buffer in a domestic microwave oven at full power (750 W) for 15 minutes, then the samples were immersed in methanol containing 0.3% H_2O_2 to inactivate endogenous peroxidase at 37°C for 30 minutes. To eliminate nonspecific staining, we incubated the slides with appropriate preimmune serum for 30 minutes at room temperature, followed by incubation at 4°C overnight with following primary antibodies: β-catenin (clone 14, mouse monoclonal, 1:300 dilution; Transduction Labs, Becton Dickinson, San Diego, CA), COX-2 (Entrez-Gene ID #5743; rabbit polyclonal COX-2 antibodies #4842; Cell Signaling Technology, Inc., Boston, MA; 1:200 dilution), cyclin D1 (Clone EP12; rabbit monoclonal anti-human, Cyclin D1, Epitomics, Inc., Burlingame, CA; 1:200 dilution), c-Myc (Gene ID 4609, mouse monoclonal(9E10) sc-40, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; 1:200 dilution), E-cadherin (Entrez-Gene ID #999; rabbit polyclonal #4065; Cell Signaling Technology, Inc.; 1:250 dilution), and labeled polymer-horseradish peroxidase (HRP) was added according to the manufacturer's instructions and incubated for 30 minutes. Color reaction was developed by using 3-amino-9-ethylcarbazole chromogen solution. All slides were counterstained with hematoxylin. Positive control slides were included in every experiment in addition to the internal positive controls. The