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Loss of N-cadherin is associated with loss of E-cadherin expression and poor outcomes of liver resection in hepatocellular carcinoma

Yang-an Liu, MD,[a,b,1] *Bin-yong Liang, MD,*[a,1] *Yan Guan, BS Nurs,*[a] *Jian You, MD,*[c] *Ling Zhu, MD,*[b] *Xiao-ping Chen, MD,*[a] *and Zhi-yong Huang, MD*[a,*]

[a] *Research Laboratory and Hepatic Surgery Center, Department of Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

[b] *Department of Hepatobiliary and Pancreatic Surgery, Wuhan Central Hospital, Wuhan, China*

[c] *Department of General Surgery, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

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ABSTRACT

Background: Our previous study suggested that N-cadherin was downregulated in hepatocellular carcinoma (HCC). Our aim in this study was to investigate the correlation between N- and E-cadherin expression in HCC and its clinical significance.

Methods: Eighty-six patients with HCC undergoing liver resection were retrospectively studied. N- and E-cadherin expression in HCC and adjacent liver tissue were investigated using immunohistochemistry and immunofluorescence. The correlation between the expression status of both cadherins and surgical outcomes was analyzed.

Results: In 23 patients negative for E-cadherin expression, 19 of them (82.6%) were also negative for N-cadherin expression. In 30 patients with heterogeneous expression of E-cadherin, 20 of them (66.7%) also had heterogeneous expression of N-cadherin. In 33 patients with uniformly positive expression of E-cadherin, 19 of them (57.6%) also had uniformly positive expression of N-cadherin. Therefore, there was a positive correlation between expression patterns of N- and E-cadherins. Concurrent loss of both N- and E-cadherin expressions was significantly associated with absence of the tumor capsule, vascular invasion, and poor differentiation. The 1- and 3-y disease-free survival rates were 27% and 9%, respectively, and the 1- and 3-y overall survival rates were 64.3% and 14.3%, respectively, in patients with concurrent loss of both cadherins, which were significantly worse than those with concurrent uniformly positive expression or heterogeneous expression of both cadherins.

Conclusions: Loss of N-cadherin was positively correlated with loss of E-cadherin in HCC. Concurrent loss of both N- and E-cadherin expressions was associated with poor surgical outcomes of HCC patients undergoing liver resection.

* *Corresponding author.* Research Laboratory and Hepatic Surgery Center, Department of Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095, Jie Fang Da Dao, Wuhan 430030, China. Tel.: +86 27 83665392; fax: +86 27 83663432.

E-mail address: zyhuang@medmail.com.cn (Z.-y. Huang).

[1] These authors (Y.-a.L and B.-y.L.) equally contributed to this work.

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1. Introduction

Hepatocellular carcinoma (HCC), which develops typically from chronic liver disease resulting from viral hepatitis infection (hepatitis B or C), is one of the most common malignancies in the world. It has become the second most prevalent cause of cancer-related deaths in China, with about 344,000 deaths per year [1,2]. Both liver resection and transplantation are potentially curative modalities for HCC. However, most HCC patients usually lose the opportunity for surgery because of portal vein spreading or distant metastasis [3,4]. For those who have a chance to undergo a curative liver resection, long-term survival remains unsatisfactory because of postoperative intrahepatic recurrence or metastasis. The 5-y recurrence rates after liver resection have been reported to be as high as 80% [5]. Studies also suggested that approximately two-thirds of recurrences occurred in the first 18 mo after treatment (early recurrence), which had been considered a recurrence because of intrahepatic metastasis [6]. Therefore, to develop more effective treatments for HCC in the future, it is vital to further understand the molecular mechanisms underlying metastasis of HCC.

Metastasis is believed to be a complex, multistep process that involves the detachment of cancer cells from the primary tumor mass and the development of new foci in a remote organ [7,8]. Cadherins, a large family of cell adhesion molecules, mediate cell–cell interactions through a calcium-dependent, homophilic protein–protein interaction [9,10]. Cadherins have been reported to play an important role in the tumor metastatic process [10]. E-cadherin, a member of the classic cadherin family, is frequently associated with dedifferentiation and invasion in a variety of human malignancies, including HCC [11]. Reduced E-cadherin expression has been reported in numerous carcinomas originating from epithelial cells including gastric, breast, pancreatic, and hepatic, and its downregulation is frequently related to metastasis and invasiveness [12,13]. Reduced expression of E-cadherin induces cell mobility and promotes tumor cell invasion via the Wnt and/or wingless signal transduction pathway by freeing β-catenin, which travels to the nucleus and stimulates the expression of transcription factor-regulated genes such as c-Myc, cyclin D1, fibronectin, and matrilysin [13].

The role of E-cadherin in tumor invasion and metastasis has been well established in HCC [14]. However, the role in tumor pathogenesis and metastasis of another classical cadherin, N-cadherin, remains controversial. Some studies indicated that aberrant N-cadherin expression was correlated with a morphologic change toward a more fibroblastic phenotype, with tumor cells becoming more motile, invasive, and metastatic [15]. N-cadherin has often been found in neural tissues and fibroblasts and has been shown to be frequently upregulated in breast and prostate cancer, as well as in melanoma [16–18]. On the other hand, it has been reported that N-cadherin was downregulated in osteosarcoma, ovarian carcinoma, glioblastoma, and renal cell carcinoma [19–22]. These studies indicated that N-cadherin might behave as a tumor suppressor in these particular tumors. In HCC, Cho *et al.* [23] reported that discontinuous N-cadherin staining predicted a high risk of recurrence after liver resection for HCC. However, the study by Seo *et al.* [24] suggested that N-cadherin was upregulated in HCC compared with adjacent noncancerous liver tissue and significantly associated with postoperative recurrence of HCC. In our previous experimental study, we found that knockdown N-cadherin in HCCLM3 cells resulted in decreasing cell aggregation and increasing cell migration and invasion. Furthermore, downregulation of N-cadherin in human HCC was also significantly associated with poor surgical outcomes [25]. Our study suggested that, just like E-cadherin, N-cadherin plays a role as a tumor suppressor in HCC. However, the relationship between E-cadherin and N-cadherin expressions is still unclear in HCC.

In this study, our aim was to determine whether a correlation exists between E- and N-cadherin expressions in human HCC using immunohistochemistry and immunofluorescence, and to further evaluate the clinical significance of the correlation of E- and N-cadherin expressions.

2. Material and methods

2.1. Patients and specimens

HCC specimens were obtained from 86 patients, each with a single HCC smaller than 5 cm in diameter, who underwent curative resection at the Hepatic Surgery Center, Tongji Hospital, Huazhong University of Science and Technology between 2002 and 2009. The diagnosis of HCC was confirmed by pathology. Patient ages ranged from 28–75 y, with an average age of 48 y. There were 77 males and 9 females, 66 of whom were found to have liver cirrhosis. Tumor differentiation was determined according to the World Health Organization International Histologic Classification. A total of 20 patients had well-differentiated, 47 patients had moderately differentiated, and 19 patients had poorly differentiated tumors. Of these, 24 patients were found with a portal vein tumor thrombus through preoperative imaging studies and postoperative pathologic examinations. Follow-up data after liver resection were available for all 86 patients. The median follow-up was 36 mo. During follow-up, the serum alpha-fetoprotein test, abdominal ultrasound, and chest radiographic examination were performed once in 2 mo, and computed tomography and/or magnetic resonance imaging examinations were used if recurrence or metastasis was suspected. For intrahepatic recurrence, radiofrequency or microwave ablation, ethanol injection, and transcatheter arterial chemoembolization were performed. The Medical Ethics Committee of Tongji Hospital approved this study. Informed consent was obtained from each patient.

2.2. Immunohistochemistry assay

Four-micrometer-thick sections were prepared from paraffin-embedded specimens. After deparaffinization and rehydration, endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol solution. Sections were then processed to unmask antigens by placing them in a microwave oven and heating in citrate buffer for 15 min (pH 6.0). Sections were then incubated overnight at 4°C with an E-cadherin (1:100, BD,