The American Journal of Surgery (2014) 208, 450-456

The American Journal of Surgery®

Clinical Science

Age-related clinicopathologic and molecular features of patients receiving curative hepatectomy for hepatocellular carcinoma

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KEYWORDS:
Hepatocellular carcinoma;
Aged;
Gene expression;
Fibrosis;
Pre-existing comorbidity;
Non-B non-C hepatocellular carcinoma

Abstract

BACKGROUND: Age-related differences of clinicopathologic features, outcomes, and molecular properties of hepatocellular carcinoma remain unclarified.

METHODS: We classified patients who underwent hepatectomy for hepatocellular carcinoma into 3 groups by age bracket; younger group (<50 years), middle-aged group (50 to 79 years), and elderly group (≥80 years) and compared age-related features.

RESULTS: Hepatitis viral infection was dominant in the younger group (hepatitis B virus [HBV]; 67%) and middle-aged group (hepatitis C virus [HCV]; 56%), whereas the elderly group showed a significantly higher rate without hepatitis virus infection (absence of HBV and HCV infection, 66%; $P = .0001$). There was a significantly greater proportion of age-associated pre-existing comorbidity in the elderly group (89%; $P = .0004$). Liver cirrhosis in the elderly group (24%) was significantly lower than other groups (younger, 67%; middle-aged, 50%; $P = .0058$). There was no significant difference in perioperative and postoperative outcomes among these groups. Microarray analysis revealed age-related upregulation of androgen and phosphatidylinositol 3-kinase pathways in the tumor tissue and downregulation of the fibrosis-related pathways in the noncancerous liver tissue.

This work was supported by Grant-in-Aid for Scientific Research on Innovative Areas, Scientific Research (A), Project of Development of Innovative Research on Cancer Therapeutics from Ministry of Education, Culture, Sports, Science & Technology of Japan, and Health & Labour Sciences Research Grant from Ministry of Health Labour & Welfare of Japan.

The authors declare no conflicts of interest.

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Manuscript received December 6, 2013; revised manuscript December 24, 2013

0002-9610/$ - see front matter
http://dx.doi.org/10.1016/j.amjsurg.2014.01.015

CONCLUSIONS: Based on increased correlation with the absence of HBV and HCV infection and pre-existing comorbidity, the age-related carcinogenic pathways might play a critical role in elderly hepatocarcinogenesis.

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and one of the most common causes of cancer-related deaths in the world.[1,2] Recently, as the population is aging, the number of elderly HCC patients is increasing in the developed countries including Japan.[3] Although surgical resection is considered one of the main curative treatments of HCC,[4,5] the perioperative and postoperative outcomes in the elderly HCC patients have been controversial.[6–11] As the previous study suggested some different steps or mechanisms of hepatocarcinogenesis according to the patient's age-distribution,[12] the difference of pathologic features and molecular properties should be clarified.

In this study, we classified the patients who underwent hepatectomy for HCC into the 3 groups by age bracket, and compared the clinicopathologic features among the groups. Then, the perioperative morbidities were evaluated by Clavien-Dindo grading system, and postoperative outcomes were evaluated recurrence-free and overall survivals. Additionally, the genome-wide gene expression correlated with aging was analyzed by deoxyribonucleic acid (DNA) microarray that offers a systematic approach to acquire comprehensive information regarding gene transcription profiles.[13] Our study identified a strong correlation of aging with non-viral status and specific gene expression in the elderly HCC.

Methods

Patients and samples

We enrolled 486 patients who underwent curative hepatectomy for HCC at the Tokyo Medical and Dental University Hospital between April 2000 and February 2012. Written informed consent was obtained from these patients, and the institutional review board approved this study (#1080). The patients were classified into 3 groups by age bracket at the time of operation: younger group (<50 years), middle-aged group (50 to 79 years), and elderly group (≥80 years). We compared background characteristics, liver function data, tumor factors, perioperative outcomes, disease-free survival, and overall survival among these groups. With respect to personal lifestyles and societal conditions associated with economic development in elderly patients, we defined diabetes mellitus, hypertension, and dyslipidemia as an age-associated pre-existing comorbidity.[14]

DNA microarray analysis

The tissue preparation was essentially compliant with the General Rules for the Clinical and Pathological Study of Primary Liver Cancer. Total ribonucleic acid (RNA) was extracted from the HCC specimens with RNeasy kit (Qiagen, Hilden, Germany). The integrity of the RNA obtained was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). In this study, 178 tumor and 118 noncancerous liver tissues were available for analysis of gene expression. Contaminating DNA was removed by digestion with RNase-free DNase (Qiagen), and with 2 μg of total RNA, complementary RNA was prepared with a 1-cycle target labeling and control reagents kit (Affymetrix, Santa Clara, CA). The hybridization and signal detection of the Human Genome U133 (HG-U133) Plus 2.0 arrays (Affymetrix) were performed in accordance with the manufacturer's instructions. A total of 296 microarray data sets were normalized by the robust multiarray average method (R statistical software version 2.12.1 and the BioConductor package), essentially as described in our previous report.[15] The estimated gene expression levels were log 2 transformed, and a Pearson correlation test was performed to estimate the significance levels of the association between gene expression pattern and age. To remove genes with low variance across the samples, interquartile range (IQR) was calculated for each probe set. In tumor tissue, genes sets satisfying both $P < .001$ and IQR > 2 and in noncancerous tissue gene sets satisfying both $P < .01$ and IQR > 1.5 were considered as significant. The gene expression changes of 78 probe sets in the tumor tissue and 27 probe sets in the noncancerous tissue were evaluated in order of the P values and IQR.

Statistical analysis

Statistical comparisons of the clinicopathologic characteristics for significance were performed by the chi-square test or the Fisher exact test with a single degree of freedom, and a Student t test was used to analyze the differences between continuous values. Overall survival and disease-free survival were determined by the Kaplan-Meier method, and for comparisons, log-rank tests were used. P values less than .05 were considered to have statistical significance.

Results

Classification of patients with hepatocellular carcinoma

In this study, 486 patients who underwent curative hepatectomy for HCC were classified into 3 groups by age bracket: 24 cases in the younger group (<50 years), 433 cases in the middle-aged group (50-79 years), and the remaining 29 cases in the elderly group (≥80 years). The