Abstract. Long non-coding RNAs (lncRNAs) have been investigated as a novel class of regulators of cellular processes, including cell growth, apoptosis and carcinogenesis. lncRNA BRAF-activated non-protein coding RNA (BANCR) has recently been revealed to be involved in tumorigenesis of numerous types of cancer, including papillary thyroid carcinoma, melanoma, non-small cell lung cancer and colorectal cancer. However, the expression profiles and biological relevance of lncRNA BANCR in hepatocellular carcinoma (HCC) has not yet been reported. In the present study, the expression level of BANCR in tumor tissues and para-cancerous tissues was determined by reverse transcription-quantitative polymerase chain reaction in patients with hepatitis B virus (HBV)-associated HCC, and its association with clinicopathological characteristics of patients was analyzed. The results demonstrated that the expression level of BANCR was significantly reduced in tumor tissues in comparison with in para-cancerous tissues (P<0.001). Furthermore, the present study demonstrated that BANCR expression level was closely associated with serum α-fetoprotein levels (P<0.01) and HCC tumor number (P<0.05). To the best of our knowledge, these results revealed for the first time that BANCR downregulated in patients with HBV-associated HCC and BANCR expression level may be a potential valuable diagnosis and therapeutic biomarker in HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and aggressive human malignancies worldwide (1). The majority of the cancer burden (85%) is present in developing countries, with a particularly high prevalence in regions where hepatitis B virus (HBV) infection is endemic, including South-East Asia and Sub-Saharan Africa (2). The majority of HCC remains incurable once it has become metastatic and has a poor prognosis, although there have previously been advances in cancer treatment with respect to surgery, chemotherapy and biological therapy (3). The major causes of HCC are viral infections, alcohol and tobacco use and HBV infection (4). Despite efforts made to identify appropriate prognostic markers for HCC, including primary tumor size, elevated α-fetoprotein (AFP) levels and gene expression markers in the primary tumor, these methods have not proven adequate to predict the prognosis of all patients with HCC (5,6). Therefore, a reliable clinical biomarker for HCC diagnosis and prediction of clinical outcome is urgently required.

Long non-coding RNAs (lncRNAs) are >200 nucleotides in length and do not code for proteins but interact with them (7). Although lncRNAs are not as well characterized as small non-coding microRNAs, lncRNAs are critical in the regulation of mechanisms underlying cellular processes, including stem cell pluripotency, cell growth, cell cycle, apoptosis, metabolism and cancer migration (8-13). Functional lncRNAs can be used for cancer diagnosis and prognosis, in addition to serving as potential therapeutic targets. Tang et al (14) identified three lncRNAs, RP11-160H22.5, XLOC_014172 and LOC149086, which were upregulated in HCC tissues in comparison with in the cancer-free controls of their study. Furthermore, XLOC_014172 and LOC149086 were confirmed to be highly expressed in patients with metastatic HCC (14). The majority of patients demonstrated a decreased expression level of the three lncRNAs following surgery. In
was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer’s protocol. The RNA integrity was evaluated using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). cDNA from all samples was synthesized from 50 ng total RNA from each sample. BANCR expression levels were quantified by RT-qPCR, which was performed using the ABI7500 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and Thermo Fisher Scientific Maxima SYBR® Green qPCR Master kit (Thermo Fisher Scientific, Inc.), according to the manufacturer’s protocol. Briefly, reactions were performed in a 20 µl mixture containing 100 ng cDNA template, 10 µl 2X PCR Mix and 0.5 µl each of forward and reverse primers. The thermocycling conditions used were as follows: 95°C for 10 min, followed by 25 cycles of 95°C for 10 sec, 60°C for 30 sec and 72°C for 30 sec. RT-PCR was performed in duplicate for each sample. The expression level of GAPDH was also detected as the endogenous control, and all the samples were normalized to human GAPDH. The primer sequences used in the present study were as follows: BANCR forward, 5'-ACAGGACTCCATGGCAAACG-3' and reverse, 5'-ATG AAGAAPGCCTGGTGCAAGT-3'; and GAPDH forward, 5'-CAGCCTCAAGATCATAGCA-3' and reverse, 5'-TGT GGTCAATGTCATTCCA-3'. The median from experiments performed in triplicate was used to determine relative lncRNA concentrations (ΔCq=Cq median lncRNAs-Cq median GAPDH). Expression fold changes were calculated using the 2^ΔΔCq method (25).

**Statistical analysis.** Statistical significances between groups were determined by two-tailed Student’s t-test. A receiver operating characteristics (ROC) curve was plotted to determine how well the expression level of BANCR discriminated between HBV HCC tumor tissues and para-cancerous tissues. The association between BANCR expression level and clinicopathological characteristics was analyzed using one-way analysis of variance with Bonferroni correction. All statistical analyses were performed using SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**lncRNA BANCR is downregulated in HBV-associated HCC tissues compared with in para-cancerous tissues.** To assess the potential clinical significance of BANCR, its expression levels in HCC tissues and para-cancerous tissue samples were analyzed. Firstly, the expression level of BANCR in HBV HCC tissues samples and para-cancerous tissues samples were determined by RT-qPCR. The results demonstrated that the expression levels of BANCR were markedly decreased in HCC tissues compared with in para-cancerous tissues (P<0.001; Fig. 1A). Subsequently, ROC analysis was used to evaluate the suitability of BANCR expression level to discriminate between the tumor and control tissue samples. Total area under the curve (AUC) for BANCR was 0.76 (Fig. 1B), which suggested that BANCR has adequate sensitivity and specificity to discriminate between HCC and para-cancerous tissue samples. These results
revealed that the expression levels of BANCR significantly decreased in HBV-associated HCC tissues compared with in para-cancerous tissue samples.

**IncRNA BANCR expression level is associated with the AFP levels and tumor numbers in patients with HBV-associated HCC.** To investigate whether the expression levels of IncRNA BANCR in the HBV-associated HCC tissues are associated with the disease clinicopathological parameters, the present study analyzed the expression profiles of BANCR in tumor tissue samples according to AFP levels and tumors numbers. Since serum AFP level is a critical tumor biomarker for patients with HCC, the evidence resulted in the investigation of whether there is any association between BANCR expression level and serum AFP concentration status in patients with HBV-associated HCC. As presented in Fig. 2A, the BANCR expression level was lower in the tissue samples with serum AFP ≥20 ng/ml compared with in tissue samples that had serum AFP <20 ng/ml from patients with HBV HCC. In addition, the present study compared the BANCR expression level and solitary or multiple tumor numbers in patients with HBV-associated HCC (Fig. 2B); these results revealed that the BANCR expression level and tumor numbers were positively associated in these patients.

**Association between BANCR expression level and the clinicopathological parameters of patients with HBV-associated HCC.** To further analyze the association between IncRNA expression level and various clinicopathological parameters of patients with HBV HCC, 46 patients were divided into high (n=23) and low (n=23) BANCR expression level groups, according to the mean value of the expression levels of BANCR in tumor tissue samples. As presented in Table I, clinicopathological factors were investigated between the two groups and the low BANCR expression level group demonstrated significantly higher serum AFP levels (P=0.008) and more advanced tumor numbers (P=0.047) compared with the high BANCR expression level group. However, no significant association was revealed between BANCR expression level and other clinicopathological features, including age, gender, tumor size, clinical stage and liver cirrhosis (P>0.05).
Discussion

With advancements in genome wide sequencing and high-resolution microarray technology, more attention has been received by lncRNAs (26-28). A previous study revealed that dysexpression of lncRNAs is associated with various types of disease (29). Furthermore, lncRNAs have been identified to perform an important function in the progression of various types of cancer. Numerous well-studied lncRNAs, including Hox transcription antisense RNA (30-32), maternally expressed 3 (33) and LOC285194 (34) have been reported to be strongly associated with survival of patients with cancer; thus, they have been determined to be prognostic factors for specific types of cancer.

LncRNA BANCR was first revealed to have a potential functional role in melanoma cell migration (22). Other previous studies also investigated the expression levels and functions of BANCR in various malignancies. BANCR has been demonstrated to be upregulated in numerous types of solid tumors, including papillary thyroid cancer (23) and CRC (21). Therefore, the present study hypothesized that BANCR also had similar effects on the tumorigenesis, development and progression of HCC. To confirm these hypotheses, the present study firstly determined the BANCR expression levels in 46 pairs of HBV-associated HCC tissues and their para-cancerous tissues by RT-qPCR. HCC para-cancerous tissues always demonstrate liver cirrhosis or chronic inflammations and fibrosis (35); thus, para-cancerous tissues were used as the control in the present study. It was revealed that BANCR was significantly downregulated in HBV-associated HCC and this differs from the overexpression of BANCR observed in melanoma tissue samples. The results of the present study may provide evidence for the tissue specificity of lncRNA. However, the underlying molecular mechanisms of lncRNA BANCR require further investigation. Only HBV-associated HCC patients were included in the present study and it was demonstrated that the expression level of BANCR in HBV-associated HCC tissues were lower compared with in para-cancerous tissues. Furthermore, the ROC curve analysis was performed in order to identify the diagnostic significance of BANCR expression level in HBV-associated HCC and the AUC area was 0.76, revealing a possible diagnostic value of BANCR. However, a considerably larger cohort of patients with HBV-associated HCC is required to better support these findings.

Aberrant expression level of BANCR was previously reported to be involved in the progression of numerous tumors and can be used as a prognostic indicator (36). Subsequently, the present study described the association between BANCR expression level and various clinicopathological parameters. The results also demonstrated positive associations between the BANCR expression level and tumor number and serum AFP value. As a diagnostic indicator, AFP serves an essential role in the diagnosis of HCC and there was a significant association between BANCR expression level and serum AFP in the present study. Thus, BANCR may be a biomarker for the occurrence of HBV-associated HCC. However, no significant association was identified between the BANCR expression level and other clinic pathological features, including age, gender, tumor size, clinical stage and liver cirrhosis. Further \textit{in vitro} and \textit{in vivo} experiments are presently being performed to investigate the biological function and molecular basis of lncRNA BANCR expression in patients with HCC.

In conclusion, the results of the present study demonstrated, to the best of our knowledge, for the first time that lncRNA BANCR expression level was significantly lower in HBV-associated HCC tissues, and downregulation of lncRNA BANCR was positively associated with serum AFP level and tumor number in patients with HBV-associated HCC. These results suggested that lncRNA BANCR may be a potential novel diagnosis biomarker for HBV-associated HCC.

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Table I. Association between BANCR expression and clinicopathological characteristics of patients with HBV-associated HCC.

| Parameters          | Total, n | Low, n | High, n | P-value |
|---------------------|----------|--------|---------|---------|
| Gender              |          |        |         |         |
| Male                | 40       | 19     | 21      | 0.665   |
| Female              | 6        | 4      | 2       |         |
| Age                 |          |        |         |         |
| <60 years           | 36       | 17     | 19      | 0.475   |
| ≥60 years           | 10       | 6      | 4       |         |
| Tumor size          |          |        |         |         |
| <5 cm               | 25       | 12     | 13      | 0.767   |
| ≥5 cm               | 21       | 11     | 10      |         |
| AFP                 |          |        |         |         |
| <20 ng/ml           | 21       | 15     | 6       | 0.008*  |
| ≥20 ng/ml           | 25       | 8      | 17      |         |
| Histological grade  |          |        |         |         |
| Well                | 41       | 20     | 21      | 0.349   |
| Moderately-poorly   | 5        | 3      | 2       |         |
| TNM stage           |          |        |         |         |
| I-II                | 35       | 19     | 16      | 0.491   |
| III                 | 11       | 4      | 7       |         |
| Liver cirrhosis     |          |        |         |         |
| Absence             | 33       | 15     | 18      | 0.326   |
| Presence            | 13       | 8      | 5       |         |
| Tumor number        |          |        |         |         |
| Solitary            | 38       | 22     | 16      | 0.047*  |
| Multiple            | 8        | 1      | 7       |         |

Table I summarizes the association between BANCR expression level and clinicopathological features in patients with HBV HCC. *P<0.05; **P<0.01. BANCR, BRAF-activated non-protein coding RNA; AFP, α-fetoprotein; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TNM, tumor-node-metastasis.
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