Plasma LncRNA BANCR as a Novel Biomarker for Diagnosis and Prognosis of Hepatocellular Carcinoma in Egyptian Patients

Amal Zidan [1], Nashwa M Alazizi [1], Maysaa Abdallah Saeed [2], Dina Mostafa Hamed [1*], Amany M Sediq [1]

1 Clinical Pathology Department, Faculty Of Medicine, Zagazig University, Egypt

2 Endemic and Tropical Medicine, Faculty Of Medicine, Zagazig University, Egypt

Corresponding author: Dina Mostafa Hamed

E-mail: Dinamostafa2030@gmail.com, dmatia@medicine.zu.edu.eg

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Abstract

Background: We aimed to evaluate the effectiveness of long non coding RNA BANCR (LncRNA BANCR) as biochemical marker in diagnosis and prognosis of Hepatocellular carcinoma (HCC).

Methods: The present study was conducted on 126 Egyptian individuals divided into 3 groups, 42 patients with HCC, 42 patients with benign liver diseases, and 42 healthy individuals as normal controls. They were subject to full history taking, full clinical and laboratory examination, and assessment of LncRNA BANCR level by real-time PCR.

Results: A statistically significant difference was found with $p< 0.01$ between HCC and each of benign and control groups as regards BANCR level. Increased plasma BANCR expression predicted unfavorable overall survival in HCC independently. Furthermore, ROC curve analysis confirmed plasma BANCR as a useful marker in discriminating HCC from benign liver diseases and healthy controls.

Conclusions: These findings suggested that plasma BANCR expression was upregulated in HCC and might act as a novel diagnostic and prognostic biomarker.

Keywords: LncRNA, BANCR, hepatocellular carcinoma, biomarker, diagnosis, prognosis.

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Introduction:

Hepatocellular carcinoma (HCC) is the most prevalent cancer in Egypt, and the fifth most common disease worldwide (1). This exceptional circumstance is mainly explained by the unusual scope of the Hepatitis C Virus (HCV) epidemic in the country (2), which has historically been linked to the widespread parenteral anti-schistosomal treatment campaigns in the 1960s -1970s (3) and more recently, risky medical practices (4).

The major causes of HCC are viral infections, alcohol and tobacco use (5). Despite efforts to find suitable prognostic indicators for HCC, such as initial tumor size, high fetoprotein (AFP) levels, and gene expression markers in the primary tumor, these approaches have not been found

to be sufficient to forecast the prognosis of all HCC patients (6,7). Therefore, a reliable clinical biomarker for HCC diagnosis and prediction of clinical outcome is urgently required.

It was shown that long non-coding RNAs (lncRNAs), transcriptional products of the eukaryotic genome with lengths longer than 200 nucleotides and limited capacity for protein coding, have important roles in a number of biological processes and disease states (8-10). lncRNAs play a role in a variety of biological functions, and their dysregulation of lncRNAs has been linked to numerous human disorders, including cancer (11).

LncRNAs are essential for the development, growth, and metastasis of tumors (12,13). Furthermore, being detectable and stable in serum, plasma, urine, and other bodily fluids make them a novel avenue for the search of tumor biomarkers (14,15).

LncRNA BANCR (BRAF-activated non-coding RNA), 693 bp in length and located in chromosome 9, is a newly discovered cancer-related lncRNA. Numerous studies have demonstrated aberrant BANCR expression and its tumor suppressive or oncogenic function in a variety of cancers, including thyroid (16), non-small cell lung (17), bladder (18), melanoma (19, 20), colorectal (21), gastric (22), endometrial (23), esophageal squamous cell carcinoma (ESCC) (24), and HCC (25).

In HCC tissues, BANCR expression was markedly upregulated, and this upregulation was associated with aggressive clinicopathological characteristics and decreased overall survival [25].

Thus, the identification of novel, reliable, and non-invasive biomarkers for HCC allows a detailed understanding of the molecular mechanisms underlying hepatic carcinogenesis, as well as providing a tool for early diagnosis, which improves patients' outcomes. So, we conducted this study to examine the possibility of relying on a new early biomarker in hepatocellular cancer patients to be used for early diagnosis.

Subjects and Methods:

This prospective case-control study was conducted in Clinical Pathology and Tropical Medicine Departments - Zagazig University Hospitals. Samples collection started in November 2019 and ended in April 2021, and patients were followed up for one year after the initial diagnosis (for later calculation of overall survival). The study was approved by The Zagazig University Institutional Review Board (ZU-IRB:4345/11-2-2018) in accordance with the Helsinki Declaration. Informed written consents were gathered from all patients before enrolling them in the study.

A total of 126 subjects were included in the study, divided into 3 groups: (1) control group: 42 healthy subjects, (2) benign liver disease group: 42 patients, and (3) HCC group: 42 patients newly diagnosed as HCC by imaging studies (abdominal sonography, computed tomography or magnetic resonance imaging). All subjects were age and gender matched. Patients who had history of prior exposure to radiation, chemotherapy, or carcinogens, patients with HCC

on follow up, patients with other malignancy, and patients who refused to participate in the study were all excluded.

All individuals underwent a full medical history, clinical examination, abdominal ultrasonography, and the required laboratory investigations as follow:

For each individual, three blood samples were collected as follows. One blood sample (3 ml) was collected on plain vacutainer tube (red-topped) for serum separation that was used to test for (total and direct bilirubin, ALT, AST, ALP, albumin, creatinine, urea, and AFP). Analysis was performed using dedicated reagents on Cobas 8000/c702&e602 (Roche Diagnostics, Mannheim, Germany). Two blood samples (2 ml each) were collected on EDTA vacutainer tubes (lavender-topped). One was used for complete blood count (CBC) on Sysmex XS autoanalyzer (Sysmex Corporation, Kobe, Japan). The other was used for LncRNA BANCR Gene Expression analysis.

LncRNA BANCR Gene Expression Analysis:

- *Sample preparation:* EDTA plasma was separated by centrifugation of EDTA whole blood tube at 2,000 g for 5 min at 4°C, followed by centrifugation at 12,000 g for 5 min at 4°C, to thoroughly remove any cell debris. All blood samples were prepared within 6 h after collection, and the plasma was stored at -80°C until further analysis.
- *Total RNA separation:* Total RNA was extracted from plasma samples using miRNeasy mini kit according to manufacturer protocol (Qiagen, Hilden, Germany). Extracted RNA was subjected to RNA quantitation and purity assessment using NanoDrop® (ND)-1000 spectrophotometer (NanoDrop technologies, Wilmington, USA).
- *Reverse transcription:* RNA reverse transcription into cDNA was carried out using miScript® II RT kit according to manufacturer protocol (Thermo Fisher Scientific, Waltham, USA). The miScript PCR system uses total RNA that contains non-coding RNA as the starting material for cDNA synthesis.
- *PCR primers:* Target-specific primers assay for LncRNA BANCR and GAPDH (as endogenous housekeeping genes) were supplied by Invetrogen (Thermo Fisher Scientific, Waltham, USA). The primer sequences used in this study were as follows: LncRNA BANCR, 5'-ACA GGA CTC CAT GGC AAA CG-3' (forward) and 5'-ATG AAG AAA GCC TGG TGC AGT-3' (reverse); GAPDH, 5'-AGA GGC AGG GATGAT GTT CTG-3' (forward) and 5'-GAC TCA TGA CCA CAG TCC ATGC-3' (reverse). qRT-PCR was carried out using miScript SYBR® Green PCR kit (Thermo Fisher Scientific, Waltham, USA).
- *Calculations:* The fold changes (*FC*) of expression for LncRNA BANCR was calculated using $2^{-\Delta\Delta Ct}$ method (26).

Statistical analysis

The collected data were computerized and statistically analyzed using Statistical Package for Social Science program (SPSS version 25) (IBM; Armonk, New York, USA). Categorical data was expressed as number and percentage, then compared using Chi Square test. For numerical

data, the Shapiro–Wilk test was used as a test for normality. Normally distributed variables were expressed as mean ± standard deviation (x±SD). Non-parametric numeric variables were expressed as median and interquartile range (IQR). One-way ANOVA test or Kruskal–Wallis test (according to type of data) was used to test for presence of significant differences between groups. Pairwise testing for variables that showed significant difference was performed using either Least Significance Test (LSD) or Mann-Whitney test (according to type of data). The bivariate Pearson correlation test (two-tailed) was used to test correlation between variables. Receiver Operating Character (ROC) curve was constructed to determine the needed cut off with the best performance. The mean value of plasma BANCR was chosen to assign the HCC patients to high plasma BANCR group or low plasma BANCR group. Kaplan-Meier analysis with the log-rank test was run to determine if there were differences in the survival distribution for the two groups. Cox regression analysis was used to detect predictors of survival within HCC group. For all tests, $P < 0.05$ was considered as a cutoff value for significance.

Results

Patient's Demographic data in the different studied groups:

Analysis of the demographic data of the studied groups showed that There was no significant difference among the studied groups as regard age and gender (Table 1).

Table (1): Demographic data among the studied groups

Variable	HCC group (n=42)	Benign group (n=42)	Control group (n=42)	test	*P* value
Age (years) (x±SD)	59.62±4.78	57.24±7.80	57.57±7.12	F= 0.780	0.463
Sex (n-%) • Female • Male	10-23.8 32-76.2	6-14.3 36-85.7	8-19 34-81	χ^2 = 0.618	0.734

(*P1*) HCC vs control, (*P2*) Benign group vs control, and (*P3*) HCC vs benign group. χ^2 = Chi square, F= ANOVA test, K= Kruskal Wallis test, (x±SD)= mean ± standard deviation.

All the laboratory parameters showed statistically significant differences between the studied groups, On the other hand total bilirubin showed no significant difference (Table 2).

Table 2. Laboratory findings in the studied groups.

Variable	HCC group (n=42)	Benign group (n=42)	Control group (n=42)	test	*P* value	Between groups
INR (x±SD)	1.33±0.30	1.12±0.15	1.02±0.04	F= 13.846	<0.001	*P1*<0.001 *P2*=0.099 *P3*=0.001
Hemoglobin (g/dl) (x±SD)	12.13±2.13	13.38±1.75	12.14±2.19	F= 2.603	0.082	*P1*=0.988 *P2*=0.054 *P3*=0.052
Platelets count (x10^3/µL) Median (IQR)	135 (84.5-218)	190 (142-263)	270 (192-315.5)	K= 12.341	0.002	*P1*<0.001 *P2*=0.095 *P3*=0.065
TLC (x10^3/µL) (x±SD)	6.77±2.76	5.91±1.68	8.43±1.75	F= 7.642	0.001	*P1*=0.014 *P2*<0.001 *P3*=0.196
Total bilirubin (mg/dl) Median (IQR)	1.12±0.66 0.9 (0.64-1.55)	0.89±0.31 0.9 (0.65-1.06)	1.04±0.14 1 (0.9-1.2)	K= 3.514	0.173	——
Direct bilirubin (mg/dl) Median (IQR)	0.50±0.47 0.29 (0.15-0.75)	0.15±0.15 0.1 (0.06-0.2)	0.17±0.06 0.2 (0.1-0.2)	K= 16.799	<0.001	*P1*=0.021 *P2*=0.075 *P3*<0.001
Albumin (g/dl) (x±SD)	3.65±0.65	4.06±0.46	4.75±0.41	24.337	<0.001	*P1*<0.001 *P2*<0.001 *P3*=0.011
ALT (u/l) Median (IQR)	39 (30-48.5)	47 (36-75.5)	16 (11-22)	K= 33.819	<0.001	*P1*<0.001 *P2*<0.001 *P3*=0.171
AST (u/l) Median (IQR)	48 (36.5-54.5)	45 (32.5-70)	23 (20.5-30)	K= 29.773	<0.001	*P1*<0.001 *P2*<0.001 *P3*=0.906
ALP (u/l) Median (IQR)	203 (190-323)	180 (104-219)	70 (67-87)	K= 40.508	<0.001	*P1*<0.001 *P2*<0.001 *P3*=0.058
BUN (mg/dl) (x±SD)	36.64±13.36	20.38±7.10	9.29±1.65	F= 51.471	<0.001	*P1*<0.001 *P2*<0.001 *P3*<0.001
Creatinine (mg/dl) (x±SD)	1.01±0.23	0.74±0.17	0.90±0.26	F= 7.486	0.001	*P1*=0.149 *P2*=0.021 *P3*<0.001
AFP (ng/ml) Median (IQR)	47.2 (12.7-1561.5)	8.3 (3.9-17.35)	2.1(1.2-4.5)	K= 33.703	<0.001	*P1*<0.001 *P2*=0.002 *P3*=0.006

(*P1*) HCC vs control, (*P2*) Benign group vs control, and (*P3*) HCC vs benign group. χ^2 = Chi square, F= ANOVA test, K= Kruskal Wallis test, (x±SD)= mean ± standard deviation, INR= International Normalized Ratio, TLC = Total Leukocytic Count, ALT = Alanine Transaminase, AST = Aspartate Transaminase, ALP = Alkaline Phosphatase, BUN= Blood Urea Nitrogen, and AFP = α-fetoprotein.

Gene expression analysis of plasma BANCR and its diagnostic criteria in HCC:

In HCC group, the plasma BANCR level was significantly higher than benign group and healthy controls (both *P*<0.001). On the other hand, No significant difference was observed in the plasma BANCR expression between benign group and healthy controls (*P=0.107)* (figure 1).

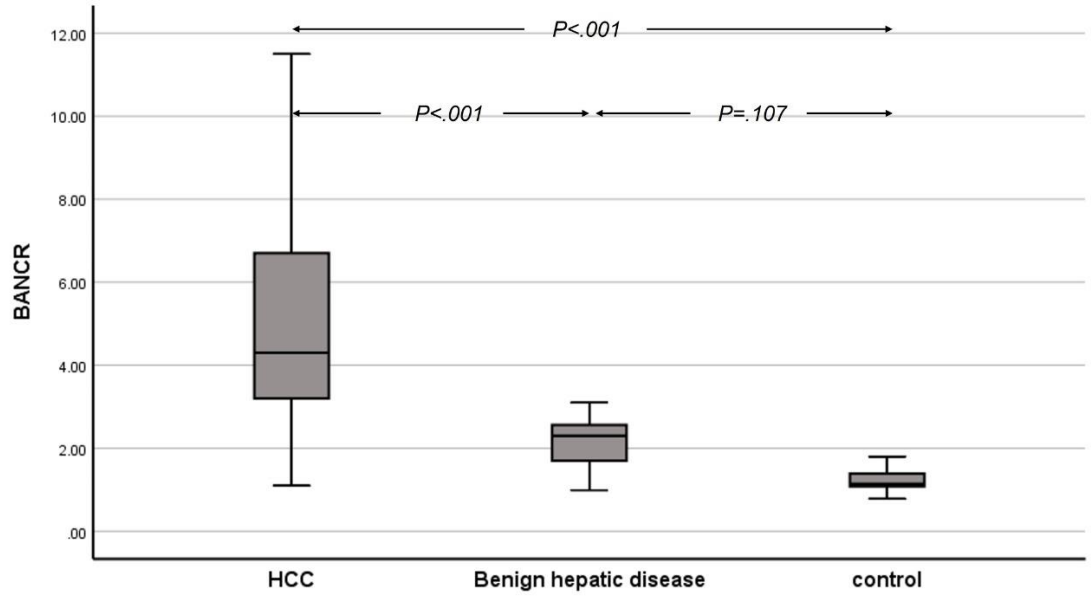

Figure (1): plasma BANCR expression (Δ Ct).

Table (3) displayed the associations between plasma BANCR levels and the clinicopathological features, There were no significant differences between plasma BANCR levels and the clinical features.

Table (3): Association between plasma BANCR levels and clinicopathological features in HCC patients:

Variable		BANCR median (IQR)	Tests	
			z	*P* value
AGE	<60 years (n=26)	4.3 (2.7-6.9)	-0.471	0.638
	>60 years (n=16)	4.9 (3.25-9.48)		
Gender	Male (n=32)	4.9 (2.53-7.08)	-0.083	0.934
	Female (n=10)	4.3 (3.5-7.85)		
Tumor size (mm)	<22 (n=20)	6.15 (2.93-10.03)	-0.881	0.379
	>22 (n=22)	4.2 (3.1-6.3)		
Child-Pugh class	Class A (n=18)	3.2 (1.55-4.75)	-2.524	0.012
	Class B (n=24)	6.45 (4.23-9.6)		
Intra-hepatic metastasis	Yes (n=10)	9.9 (2.35-10.45)	-0.826	0.409
	No (n=32)	4.25 (3.18-6.53)		
vascular invasion	Yes (n=8)	5.55 (2.7-9.23)	-0.358	0.720
	No (n=34)	4.3 (3.15-6.65)		

Roc curve analysis was performed to detect the diagnostic value of plasma BANCR for HCC. As shown in (**figure 2b**), the AUC for plasma BANCR was 0.958(95% CI: 0.892-1.000; sensitivity:

85.7%, specificity: 100%) in discriminating HCC from healthy controls, higher than that for AFP (AUC: 0.941, (95% CI: 0.873-1.000; sensitivity: 81%, specificity: 95.2%) (*P*<0.001). Moreover, plasma BANCR still had a better performance compared with AFP in discriminating HCC from benign liver diseases (**figure 2a).** the AUC for plasma BANCR was 0.845 (95% CI: 0.703-0.986; sensitivity: 81%, specificity: 90.5%), higher than that for AFP (AUC: 0.824, (95% CI: 0.961-0.958; sensitivity: 76.2%, specificity: 61.9%) (*P*<0.001).

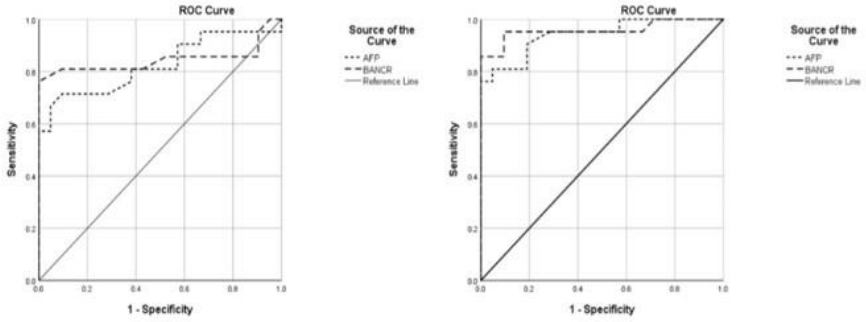

Figure 2a Figure 2b

Figure (2): ROC curve analysis of plasma BANCR versus AFP in discriminating HCC group from benign liver disease and healthy control.

Prognostic value of plasma BANCR in HCC

The median value of plasma BANCR was chosen as a cutoff and used to assign the HCC patients to high plasma BANCR group or low plasma BANCR group. Kaplan-Meier analysis with the log-rank test indicated that HCC patients in high plasma BANCR group had a significantly shorter overall survival than those in low plasma BANCR group (*P*<0.001) (Figure 3). Cox regression analysis demonstrated that plasma BANCR was a significant predictor of HCC (*P*=0.027) (table 4).

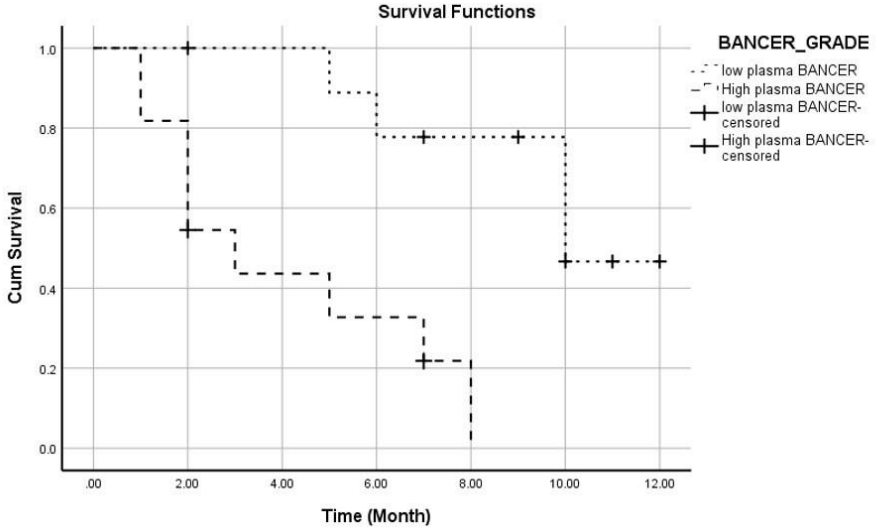

Figure (3): Overall survival curves of HCC patients.

Table (4): Cox Regression of different predictors for survival within HCC patients

Variables	Hazard ratio	95% CI		P value
		Lower	Upper	
plasma BANCER level	20.397	1.404	296.253	0.027
AFP level	4.689	1.013	21.710	0.048
Age group	0.855	0.216	3.387	0.823
Tumor size	1.363	0.312	5.948	0.680
Gender	0.567	0.117	2.745	0.481

Discussion

Hepatocellular carcinoma (HCC) is one of the most common solid tumors (27). Globally, this cancer has different prevalence throughout the world (28), with high prevalence in Egypt.(29)

Approximately 80% of hepatocellular carcinoma patients have been associated with liver cirrhosis, and even after comprehensive therapies with surgical excision, radiofrequency, or cryotherapy, this tumor shows a high percentage of recurrence and metastasis and the mean survival of the patients is still short compared to other major solid tumors, leading to death within 6 to 20 months *(30)*.

Currently, the most commonly used methods for screening and diagnosing HCC are ultrasound imaging and serum α-fetoprotein (AFP) measurements. AFP has been used worldwide as the golden standard compared to other serum markers. However, the diagnostic value of AFP is still controversial given that its sensitivity and specificity are unstable *(31)*.

LncRNAs are non-coding RNAs with more than 200 nucleotides in length, which play an important role in the regulation of gene expression. These are involved in translational regulation, alternative splicing, epigenetics, and other biological processes (32).

LncRNA BANCR, also known as BRAF-activated non-coding RNA, is a cancer-related lncRNA that has a length of 693 base pairs and can be found on chromosome 9. The level of BANCR expression was significantly elevated in HCC tissues, and its up-regulation was found to be associated with aggressive clinicopathological features and a shorter overall survival time (33).

The aim of this study is to evaluate the role of LncRNA BANCR in the diagnosis and prognosis of HCC.

In this work, we analyzed the gene expression of LncRNA BANCR and its levels was correlated with clinical and laboratory findings. Moreover, ROC curves were established and analyzed to demonstrate the diagnostic and prognostic potentials of this biomarker.

The study included 126 patients divided into three groups; Group 1 included 42 patients diagnosed with HCC (32 males and 10 females) with a mean age of 59.62+4.78 years, and Group 2 included 42 patients diagnosed with benign liver diseases (36 males and 6 females), with a mean age 57.24 _ 7.8 years. In addition, 42 healthy controls (34 males and 8 females) were included in this study with a mean age of 57.75 _ 7.8 years.

In this present study, BANCR expression levels were highly significantly increased in the HCC group compared to benign and control groups ($p< 0.001$). These results are similar to the results of **Yunsheng Qin et al.**(34) who found that The relative BANCR levels in plasma samples from HCC patients were significantly higher than those obtained from benign liver disease group and healthy controls (both P < 0.01). In addition, **JING LI et al.** (35), revealed that BANCR was overexpressed in Huh7 cells when compared with normal liver cells. The downregulation of BANCR significantly inhibited the proliferation and colony formation ability, and induced cell cycle arrest and apoptosis of Huh7 cells.

Moreover, **Xiangyang Yu et al.** (36), founded that BANCR was significantly downregulated in HCC tissues and HCC cells.

NA-NA ZHAO et al. (37), demonstrated that the expression level of BANCR was significantly reduced in tumor tissues in comparison with para-cancerous tissues (P<0.001). Furthermore, demonstrated that BANCR expression level was closely associated with serum α-fetoprotein levels (P<0.01) and HCC tumor number (P<0.05). This discrepancy may be due to different studied groups.

Regarding the correlation between BANCR and demographic and laboratory data between HCC patients and other groups, there was no significant correlation between BANCR and age, gender, tumor size, intrahepatic metastasis and vascular invasion. There was a significant difference between BANCR level child pugh class (p=0.012). The current findings were inconsistence with **Yunsheng Qin et al.** (34) who founded that increased plasma BANCR expression was significantly associated with poor tumor differentiation (p=0.005) and vascular invasion (p=0.002). These differences might be due to small sample size of the studied group.

The diagnostic value of BANCR was determined by ROC curve analysis. Discriminating HCC from benign cases, the cut-off value of BANCR was 3 with a sensitivity of 81% and a specificity of 90.5%. In discriminating HCC from healthy cases, It was found to be of diagnostic and prognostic potential for HCC at a cut-off value of 2.03 with a sensitivity of 85.7 % and a specificity of 100 % (Figure 3, 2). Our results were in agreement with **Yunsheng Qin et al. (34)** as ROC curve analyses revealed that plasma BANCR was a potential marker for discriminating HCC patients from healthy controls, with cut-off values of 0.70; sensitivity and specificity were 70.7% and 69.1%.

Future studies are encouraged to identify recent downstream genes or pathways of BANCR and the mechanisms how BANCR exerts the oncogenic function in HCC.

Recommendation: Genome-wide microarray analysis might be an ideal way to identify circulating lncRNAs with diagnostic ability, and a plasma-based biomarker panel including several lncRNAs would help to improve the sensitivity and specificity.

Conclusions

In summary, our study showed that lncRNA BANCR was significantly up-regulated in plasma samples of HCC patients, and high BANCR levels were correlated with aggressive

clinicopathological features and poor overall survival. In addition, plasma BANCR expression achieved a fine diagnostic accuracy in discriminating HCC from benign liver diseases and healthy controls. Our results suggest that plasma BANCR may act as a novel diagnostic and prognostic biomarker for HCC.

References:

1. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in egypt: results of the national population-based cancer registry program. J Cancer Epidemiol. 2014;2014: 437971. doi:10.1155/2014/437971
2. Kandeel A, Genedy M, El-Refai S, Funk AL, Fontanet A, Talaat M. The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. Liver Int. 2017;37: 45–53. doi:10.1111/liv.13186.
3. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet. 2000;355: 887–891.
4. Arafa N, El Hoseiny M, Rekacewicz C, Bakr I, El-Kafrawy S, El Daly M. Changing pattern of hepatitis C virus spread in rural areas of Egypt. J Hepatol. 2005;43: 418–424. doi:10.1016/j.jhep.2005.03.021
5. Liu YR, Tang RX, Huang WT, Ren FH, He RQ, Yang LH, Luo DZ, Dang YW and Chen G: Long noncoding RNAs in hepatocellular carcinoma: Novel insights into their mechanism. World J Hepatol 7: 2781 2791, 2015.
6. Pan K, Liang XT, Zhang HK, Zhao JJ, Wang DD, Li JJ, Lian Q, Chang AE, Li Q and Xia JC: Characterization of bridging inte¬grator 1 (BIN1) as a potential tumor suppressor and prognostic marker in hepatocellular carcinoma. Mol Med 18: 507 518, 2012.
7. Tu ZQ, Li RJ, Mei JZ and Li XH: Down regulation of long non coding RNA GAS5 is associated with the prognosis of hepa¬tocellular carcinoma. Int J Clin Exp Pathol 7: 4303 4309, 2014
8. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell 2013; 152: 1298-307.
9. Yang L, Froberg JE, Lee JT. Long noncoding RNAs: fresh perspectives into the RNA world. Trends Biochem Sci 2014; 39: 35-43.
10. Zhou S, Wang J, Zhang Z. An emerging understanding of long noncoding RNAs in kidney cancer. J Cancer Res Clin Oncol 2014; 140:
11. Zhang Y, Xu Y, Feng L, Li F, Sun Z, Wu T, Shi X, Li J and Li X. Comprehensive characterization of lncRNA-mRNA related ceRNA network across 12 major cancers. Oncotarget 2016; 7: 64148-64167.
12. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, Liu YL and Cui BB. The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/beta- catenin signaling. Mol Cancer 2017; 16: 9.

13. Li Z, Hou P, Fan D, Dong M, Ma M, Li H, Yao R, Li Y, Wang G, Geng P, Mihretab A, Liu D, Zhang Y, Huang B and Lu J. The degradation of EZH2 mediated by lncRNA ANCR attenuated the invasion and metastasis of breast cancer. Cell Death Differ 2017; 24: 59-71.
14. Tong YK and Lo YM. Diagnostic developments involving cell-free (circulating) nucleic acids. Clin Chim Acta 2006; 363: 187-196.
15. Su YJ, Yu J, Huang YQ and Yang J. Circulating long noncoding RNA as a potential target for prostate cancer. Int J Mol Sci 2015; 16: 13322- 13338.
16. Liao T, Qu N, Shi RL, Guo K, Ma B, Cao YM, Xiang J, Lu ZW, Zhu YX, Li DS and Ji QH. BRAFactivated LncRNA functions as a tumor suppressor in papillary thyroid cancer. Oncotarget 2017; 8: 238-247.
17. Sun M, Liu XH, Wang KM, Nie FQ, Kong R, Yang JS, Xia R, Xu TP, Jin FY, Liu ZJ, Chen JF, Zhang EB, De W and Wang ZX. Downregulation of BRAF activated non-coding RNA is associated with poor prognosis for non-small cell lung cancer and promotes metastasis by affecting epithelial- mesenchymal transition. Mol Cancer 2014; 13: 68.
18. He A, Liu Y, Chen Z, Li J, Chen M, Liu L, Liao X, Lv Z, Zhan Y, Zhuang C, Lin J, Huang W and Mei H. Over-expression of long noncoding RNA BANCR inhibits malignant phenotypes of human bladder cancer. J Exp Clin Cancer Res 2016; 35: 125.
19. Flockhart RJ, Webster DE, Qu K, Mascarenhas N, Kovalski J, Kretz M and Khavari PA. BRAFV600E remodels the melanocyte transcriptome and induces BANCR to regulate melanoma cell migration. Genome Res 2012; 22: 1006-1014.
20. Li R, Zhang L, Jia L, Duan Y, Li Y, Bao L and Sha N. Long non-coding RNA BANCR promotes proliferation in malignant melanoma by regulating MAPK pathway activation. PLoS One 2014; 9: e100893.
21. Guo Q, Zhao Y, Chen J, Hu J, Wang S, Zhang D and Sun Y. BRAF-activated long non-coding RNA contributes to colorectal cancer migration by inducing epithelial-mesenchymal transition. Oncol Lett 2014; 8: 869-875.
22. Zhang ZX, Liu ZQ, Jiang B, Lu XY, Ning XF, Yuan CT and Wang AL. BRAF activated non-coding RNA (BANCR) promoting gastric cancer cells proliferation via regulation of NF-kappaB1. Biochem Biophys Res Commun 2015; 465: 225- 231.
23. Wang D, Wang D, Wang N, Long Z and Ren X. Long non-coding RNA BANCR promotes endometrial cancer cell proliferation and invasion by regulating MMP2 and MMP1 via ERK/MAPK signaling pathway. Cell Physiol Biochem 2016; 40: 644-656.
24. Liu Z, Yang T, Xu Z and Cao X. Upregulation of the long non-coding RNA BANCR correlates with tumor progression and poor prognosis in esophageal squamous cell carcinoma. Biomed Pharmacother 2016; 82: 406-412.
25. Zhou T and Gao Y. Increased expression of LncRNA BANCR and its prognostic significance in human hepatocellular carcinoma. World J Surg Oncol 2016; 14: 8. 1989-95
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real time quantitative PCR and the 2-ΔΔCT method. Methods. 2001;25(4):402–408. doi:10.1006/meth.2001.1262

27. 27-Bihrer V, Waidmann O, Friedrich-Rust M, et al. Serum microRNA-21 as marker for necroinflammation in hepatitis C patients with and without hepatocellular carcinoma. PLoS One. 2011; 6: e26971.
28. Lehman EM, Soliman AS, Ismail K, et al. Patterns of hepatocellular carcinoma incidence in Egypt from a population-based cancer registry. Hepatol Res. 2008; 38: 465- 473.
29. Abdel-Atti E. HCC burden in Egypt. Gastroenterology Hepatology. 2015; 2: 45.
30. Schottenfeld D, Fraumeni JF.(2006): Incidance of hepatocellular carcinoma. In: Cancer epidemiology and prevention. 3rd ed.; 11: 170-179.
31. Liu C, Xiao G, Yan L. (2013):Value of α-fetoprotein in association with clinicopathological features of hepatocellular carcinoma World J Gastroenterol; 19(11): 1811–1819.
32. Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, et al. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature. 2012;491(7424):454-7.
33. Trzybulska, D., Vergadi, E., & Tsatsanis, C. (2018). miRNA and other non-coding RNAs as promising diagnostic markers. Ejifcc, 29(3), 221.
34. Qin y, Wu J, Ke Z, Xu J. Expression of plasma lncRNA BANCR in hepatocellular carcinoma and its diagnostic and prognostic significance 2017; 10(8):11984-11990. ISSN:1940-5901/IJCEM0053396.
35. Li J, Wang J, Zhou W, Zhang S, Le Y, He R. Downregulation of BRAF-Activated non-coding RNA suppresses the proliferation, migration and invasion, and induces apoptosis of hepatocellular carcinoma cells. Oncol Lett 2017;14:4751–7. doi:10.3892/ol.2017.6770.
36. Xiangyang Yu, Guozhi Zhang, Peng Zhao. Downregulation of a long noncoding RNA BANCR contributes to proliferation and metastasis of hepatocellular carcinoma cancer cells in vitro and in vivo 2016;9(3):3304-3312, ISSN:1936-2625/IJCEP0020733.
37. Zhao N, Wang C, CAI Cheng, Downregulation of BRAF activated non protein coding RNA in patients with hepatitis B virus associated hepatocellular carcinoma. ONCOLOGY LETTERS 15: 7794-7798, 2018. DOI: 10.3892/ol.2018.8327