Int J Clin Exp Med 2020;13(2):836-846
www.ijcem.com /ISSN:1940-5901/IJCEM0102510

Original Article

The expressions and clinical implications of long intragenic non-coding RNA-p21 and glucose transporter 1 in primary hepatocellular carcinoma

Yingyang Liao[1], Hao Huang[2], Jingrong He[2], Jindu Li[2], Hang Ye[2], Ningfu Peng[2], Jianhong Zhong[2]

Departments of [1]Nutrition, [2]Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China

Received September 19, 2019; Accepted January 3, 2020; Epub February 15, 2020; Published February 28, 2020

Abstract: Objective: To explore the expressions of long intragenic non-coding RNA-p21 (lincRNA-p21) and glucose transporter 1 (Glut-1) in primary hepatocellular carcinoma (HCC) and their clinical values. Methods: Data for a retrospective study was collected from 264 HCC patients; the relative expressions of lincRNA-p21 and Glut-1 mRNA in the HCC and adjacent normal tissues were measured, and a qualitative analysis was made for Glut-1. Results: The relative expression of lincRNA-p21 in the HCC tissues was lower than it was in the adjacent normal tissues, but the positive rate of Glut-1 and the relative expression of Glut-1 miRNA were higher (all $P<0.001$). With an increase in the size of the HCC tumors, the alpha fetoprotein (AFP) values became higher, the T stages got higher, the distant metastasis and differentiation of the lymph nodes got poorer, the relative expression of Glut-1 mRNA increased, but the relative expression of RNA-p21 decreased (all $P<0.05$). The median survival time in the Glut-1 negative patients was longer than in the Glut-1 positive patients (29 months, 95% CI: 27.659-30.341 vs 25 months, 95% CI: 24.133-25.867), and they were significantly different ($\chi^2=30.950$, $P<0.001$); the relative expression of lincRNA-p21 was lower in the Glut-1 positive patients than in the Glut-1 negative ones, and the differences were significant ($P<0.05$). The relative expression of lincRNA-p21 was negatively correlated to Glut-1 miRNA ($r=-0.256$, $P=0.017$). Conclusion: LincRNA-p21 and Glut-1 play crucial roles in the occurrence and development of HCC. Decreased lincRNA-p21 but elevated Glut-1 suggests a poor prognosis, and they can be used as biomarkers for judging the malignancy and prognosis of HCC.

Keywords: Glucose transporter 1, primary hepatocellular carcinoma, clinical implications, prognosis

Introduction

Hepatocellular carcinoma (HCC) is a common cancer, ranking fifth in morbidity and second in mortality among all cancers [1]. Every year, there are more than 700,000 newly diagnosed HCC patients and approximately 500,000-1,000,000 new deaths in the world, and over half of the deaths are in China [2]. In the past two decades, the annual morbidity and mortality of HCC have increased remarkably, and one study demonstrated that HCC is more prevalent in Asia and Africa [3]. The etiology of HCC is complicated and closely associated with environmental and genetic factors [4].

Long intragenic non-coding RNA (lnc-RNA) exists extensively in eukaryotes. The interactions between lnc-RNA and proteins can regulate the activity and location of proteins as it mediates gene transcription and activates gene expression by recruiting transcription factors into its target gene promoters, which is closely related to tumorigenesis and progression [5]. Long intragenic non-coding RNA-p21 (lincRNA-p21), a P53-dependent transcriptional target gene and potential diagnostic marker, is involved in the proliferation, cell cycle, metabolism and reprogramming of tumor cells [6]. Published studies indicate that lincRNA-p21 plays a crucial role in the regulation of glucose metabolism as decreased lincRNA-p21 expression can inhibit P53-mediated glucose metabolism in HCC patients. The p53 signaling pathway is a negative regulator of glycolysis, and it has the effect of limiting glucose uptake by inhibiting glucose transporter 1 (Glut-1) [7, 8].

Glut-1 is an important member of the glucose transporter family. It is barely expressed in normal and tumor-adjacent epithelial cells [9, 10]. Studies have shown that Glut-1 is a key factor limiting the rate of glucose transport in cancer cells, and it is overexpressed in various tumors [11-14]. Previous studies have demonstrated that Glut-1 mediation in cancer cells results in increased glucose uptake, and Glut-1 plays a crucial role in tumor formation and metastasis [15-17]. In addition, it also acts decisively in the presence and development of tumors, as tumor growth and proliferation depends on the survival response to glycolysis [18, 19]. Glut-1 overexpression in cancer tissues provides a great deal of energy for the growth and development of tumors, and it also contributes a lot to the invasion and metastasis of tumors [17, 20]. Therefore, Glut-1 has become an important target for cancer intervention. The inhibition of Glut-1 expression in osteosarcoma cells reduces glucose uptake by the tumor cells. A decrease in glucose supply makes the tumor cells unable to obtain enough energy; as a result, the tumor cells remain at the stage of less oxygen consumption or directly have programmed apoptosis. Moreover, it also reduces the capacities of invasion and metastasis of the cancer cells while it lessens their energy supply [21, 22]. We hypothesized that lincRNA-p21 could induce the abnormal expression of Glut-1 by acting on the p53 signaling pathway, thereby affecting the growth and proliferation of HCC cells. The present study was based on the correlation between the detection of lincRNA-p21 in serum and Glut-1 expression in hepatocellular carcinoma tissues and clinicopathology, and we conducted long-term follow-ups to provide basic information for the clinical prevention and treatment of HCC. Here we report as follows.

Materials and methods

General information

A total of 264 HCC patients were admitted to the Oncology Department of Affiliated Tumor Hospital of Guangxi Medical University from March 2014 to July 2015, and they were studied retrospectively. The enrolled patients included 219 males and 45 females, and they ranged in age from 26 to 70 years old (mean, 49.1±10.0 years). They all signed and provided an informed consent. This study was approved by the Hospital Ethics Committee.

Inclusion and exclusion criteria

Inclusion criteria: Patients who met all the following conditions were eligible for enrollment in this study: the HCC diagnosis and tumor-node-metastasis (TNM) classification were performed with reference to the *Standards for Diagnosis and Treatment of Primary Hepatocellular Carcinoma* (2017 Edition); the patients were between 18 and 75 years old; the patients were operated on for HCC in our hospital to collect HCC tissues and tumor-adjacent tissues, and all the collected tissue specimens were stored at -80°C in a refrigerator [23].

Exclusion criteria: Patients were ineligible for enrollment in the study if they met any of the following conditions: incomplete available clinical data, severe heart disease, liver or kidney disease, mental illness, cerebrovascular disease, inability to cooperate with the study; difficulty or inconvenience for the follow-up, other concomitant cancer or non-primary HCC.

Methods

Qualitative assay of Glut-1 in the HCC and adjacent normal tissues: The HCC Specimens and the matched adjacent normal tissues were fixed with a formaldehyde solution, dehydrated, and made to be transparent by the combined action of ethanol and xylene, and then embedded in paraffin. The embedded specimens were sliced into 2-3-μm-thick sections. The mouse anti-human Glut-1 polyclonal antibodies and kits used in the present study were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., China (Beijing, China). Streptavidin-peroxidase (SP) immunohistochemistry was applied to determine the expression of the Glut-1 protein. The specific procedures were as follows: first, the tissue specimens were hydrated with a gradient ethanol solution (Guangzhou Dingguo Biotechnology, China), and 0.01 mol/L of phosphate buffer saline (PBS) (pH 7.4) (Guangzhou Dingguo Biotechnology, China) was added dropwise to the sections until the sections were covered completely. The sections were placed on a glass slide and washed in 0.01 mol/L of PBS (pH 7.4). Subsequently, they were subjected to heat-mediated antigen retrieval at high temperature and pressure. After the addition of goat serum, primary (1:200 dilution) and secondary (1:1,500 dilution) antibodies (Guangzhou Dingguo Biotechnology, China) were added

dropwise to the sections. Twelve hours later, the DAB technique was utilized for the chromogenic reaction (Guangzhou Rainbow Biotechnology, China). Subsequently, the sections were washed with clean water, dehydrated in a gradient ethanol solution, and ultimately mounted on the slides with xylene. Glut-1 positive was defined as the percentage of positive cells observed under a microscope with five random high-power lenses, as judged by the product of the percentage of positive cells and staining intensity. The percentage of positive cells was rated as follows: 0 points, ≤1%; 1 point, 2-10%; 2 points, 11-50%; 3 points, 51-80%; and 4 points, 81-100%. The staining intensity was graded as 0 points (negative), 1 point (weakly positive), 2 points (positive) and 3 points (strongly positive). The products of the percentage of positive cells and staining intensity were rated as 0 points (negative), 1-4 points (weakly positive), 5-7 points (positive) and 8-12 points (strongly positive). Hence, the positive rate = (weakly positive + positive + strongly positive)/total number of cases [24].

The relative expressions of lincRNA-p21 and Glut-1 mRNA in HCC and the adjacent normal tissues: The HCC and adjacent normal tissues with the 2-3 mm^2 confirmed pathological sections were removed from a refrigerator at -80°C. Trizol kits (Molecular Research Center, USA) were used for this study, and the design of the forward and reverse primers was provided by Guangzhou Rainbow Biotechnology (Guangzhou, China) [25]. A reverse transcription-polymerase chain reaction (RT-PCR) was performed to reversely transcribe the miRNA to produce cDNA with the use of a reverse transcription kit (Fernentas, Canada). cDNA was used as a template to amplify the DNA. Finally, the expression levels of lincRNA-p21 and Glut-1mRNA in the HCC tissue samples were measured using quantitative PCR with fluorescent probes. The specific procedures are shown below: first, the HCC and adjacent normal tissues were fully mixed after melting. A total of 300 μL of the sample was taken out. After 1 mL of Trizol was added to the sample, the sample was placed at rest at room temperature for 5 min to realize a full pyrolysis. Trichloromethane was added at a rate of 200 μL (trichloromethane)/mL (Trizol), and a mixture thereof was shaken vigorously by hand for 15 sec and then placed at rest at room temperature for 15 min. The mixture was then centrifuged at 12,000 rpm for 15 min at 4°C. The resulting solution was divided into three layers. RNA was dissolved in the water phase, and the upper water phase was extracted and transferred to another centrifugal tube. The 75% ethanol was added at a rate of 1 mL of 75% ethanol/mL of Trizol into the centrifugal tube. Subsequently, the centrifugal tube was mildly oscillated, and the precipitation was suspended. The resulting mixture was then centrifuged at 7,500 rpm for 5 min at 4°C, followed by discarding the supernatant as much as possible. The mixture was dried at room temperature for 5-10 min, then 40 μL of DEPC-treated water was added to dissolve the precipitate. After the extraction of total RNA from the tissue cells with a Trizol kit, the concentration and purity of lincRNA-p21 and Glut-1 mRNA were tested with a ultraviolet spectrophotometer (Sigma Corporation, USA), followed by the reverse transcription of miRNA to produce cDNA using a reverse transcription kit (Fernentas, Canada). The sequences of the forward and reverse primers for lincRNA-p21 were as follows: 5'-CCCGGGCTTGTCTTTTGTT-3' (lincRNA-p21) and 5'-GAGTGGGTGGCTCACTCTTCTG-3' (lincRNA-p21); the sequences of the forward primers for Glut-1 mRNA were 5'-AACTCTTCAGCCAGGGTCCAC-3' and 5'-CACAGTGAAGATGATGAAGAC-3'. The cycling system (25 μL) included SYBR premix (2×, 12.5 μL), target gene-generated forward and reverse primers (0.5 μL, respectively), cDNA template (2.0 μL), and ddH_2O (9.5 μL). The PCR was performed under the following conditions: pre-denaturation at 94°C for 4 min, at 95°C for 40 sec, 60°C for 30 sec, and 72°C for 30 sec, for a total of 35 cycles, followed by an extension at 72°C for 1 min. Agarose gel electrophoresis was performed to determine the products generated in the PCR amplification. The relative expression level was normalized to that of the internal control U6 snRNA and analyzed using the $2^{-\Delta\Delta C(T)}$ method. Western blot was used to measure the expression of the Glut-1 protein. Finally, the relative expression levels of lincRNA-p21 and Glut-1 mRNA were measured.

Survival assessment of the patients

Overall survival (OS) was defined as the time from the beginning of chemotherapy to the

Table 1. General and baseline information

Project		Number of patients (n/%)
Age (year)	≥60	42 (15.90)
	<60	222 (84.10)
Gender	Male	219 (82.95)
	Female	45 (17.05)
Tumor size (cm)	≤2	22 (8.33)
	2.1-4.9	115 (43.56)
	≥5.0	127 (48.11)
AFP level	<40	66 (25.00)
	40-399	100 (37.88)
	≥400	98 (37.12)
T-staging	T1	80 (30.30)
	T2	47 (17.80)
	T3	114 (43.18)
	T4	23 (8.71)
T-staging	N0	248 (93.94)
	N1	16 (6.06)
T-staging	M0	235 (89.02)
	M1	29 (10.98)
Degree of tumor differentiation	Poor differentiated	70 (26.52)
	Intermediate differentiated	152 (57.58)
	High differentiated	42 (15.90)

death of the patient or the observation time points included in this study.

Statistical analysis

The data were analyzed using SPSS statistical software, version 17.0. Continuous variables were expressed as the means ± standard deviations ($\bar{x}$ ± sd). For pairwise comparisons, continuous variables conformed to a normal distribution and the homogeneity of variance were compared between the two groups using a *t* test, and denoted as t; those without a normal distribution and homogeneity of variance were tested using a rank sum test and represented as Z. For multiple comparisons, a one-way ANOVA test was used to determine the differences across the groups; if there were differences, the Tukey method was utilized for the pairwise comparisons. The count data were represented as rates/percentages (n/%) and measured using Pearson's chi-squared test and Fisher's exact probability test, and expressed as chi-squared. The survival analysis was performed using the Kaplan-Meier method and a log-rank test. *P* values less than 0.05 were considered statistically significant.

Results

General and baseline information

A total of 264 HCC patients were enrolled in the current study. Among them, 42 patients were over 65 years old, 222 patients were under 65 years old; 219 patients were male, and 45 patients were female. A total of 22 patients reported tumors ≤2 cm in diameter; 115 patients reported tumors of 2.1-4.9 cm in diameter, and 127 patients reported tumors ≥5 cm in diameter. A total of 60 patients had an alpha fetoprotein (AFP) level <40 μg/L, 100 patients had an AFP level of 40-399 μg/L, and 90 patients had an AFP level of ≥400 μg/L. For the T-staging, the patients with T1-T4 HCC were 80, 47, 114 and 23 patients, respectively; for the N-staging, the patients with no affected lymph nodes (N0) were 248, and those with one affected lymph node (N1) were 16; for the M-staging, the patients with M0 and M1 were 235 and 29 patients, respectively. Regarding the degree of tumor differentiation, 70 patients had low differentiation, 152 patients had intermediate differentiation, and 42 patients had high differentiation (**Table 1**).

Comparison of Glut-1 expression between the HCC and adjacent normal tissues

The relative expression of lincRNA-p21 in the HCC tissues was lower than it was in the adjacent normal tissues, but the positive rate of Glut-1 and the relative expression levels of Glut-1 miRNA were higher, and the differences were statistically significant (P<0.001; **Table 2**; **Figure 1**).

Comparison of the Glut-1 expression in the HCC tissues of patients with different ages and genders

There were no significant differences in the relative expression levels of lincRNA-p21, the pos-

Table 2. Comparison of the Glut-1 expressions between the HCC and adjacent normal tissues

Group	Number of patients	Relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
HCC tissues	264	0.41±0.29	38 (14.39)	226 (85.61)	1.93±0.20
adjacent normal tissues	264	1.00±0.22	223 (84.47)	41 (15.53)	0.51±0.21
F/t	-	-15.025	528.000		79.569
P	-	<0.001	<0.001		<0.001

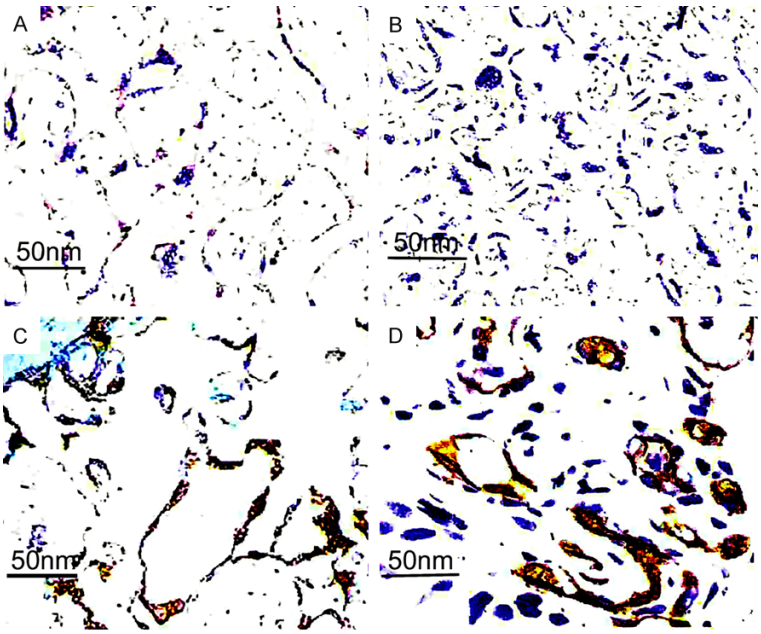

Figure 1. A comparison of the Glut-1 expression between the HCC and adjacent normal tissues. A: The negative rate of Glut-1 in the adjacent normal tissues; B: The negative rate of Glut-1 in HCC; C: The positive rate of Glut-1 in the adjacent normal tissues; D: The positive rate of Glut-1 in HCC (scale bar =50 nm).

itive rate of Glut-1, and the relative expression levels of Glut-1 miRNA in the HCC tissues of patients with different ages and genders (P> 0.05; **Tables 3** and **4**).

Comparison of Glut-1 expression in tumors of different sizes

The relative expression of lincRNA-p21 in the HCC tissues of patients with tumors ≥5 cm in diameter was lower than it was in the HCC tissues of patients with tumors of 2.1-4.9 cm and ≤2 cm in diameter, but the positive rate of Glut-1 expression and the relative expression levels of Glut-1 miRNA were higher, and there were statistically significant differences (all P< 0.001). The relative expression of lincRNA-p21 in the HCC tissues of patients with tumors 2.1-4.9 cm in diameter was lower than it was in the HCC tissues of patients with tumors ≤2 cm in diameter, but the positive rate of Glut-1 expression and the relative expression levels of Glut-1 miRNA were higher, and they were significantly different (P<0.01), as shown in **Table 5**.

Comparison of Glut-1 expressions among patients with varying AFP levels

The relative expression of lincRNA-p21 in the HCC tissues of patients with AFP values ≥400 μg/L was lower than it was in the HCC tissues of patients with AFP values of 40-399 μg/L and <40 μg/L, but the positive rate of Glut-1 and the relative expression of Glut-1 miRNA were higher, and the differences were statistically significant (P<0.05); the relative expression of lincRNA-p21 in the HCC tissues of patients with AFP levels of 40-399 μg/L was lower than it was in the HCC tissues of patients with AFP levels <40 μg/L, but the positive rate of Glut-1 and the relative expression of Glut-1 miRNA were higher, and there were statistically significant differences (P<0.05; **Table 6**).

Comparison of the Glut-1 expressions in patients at different TNM stages

The relative expression of lincRNA-p21 in the T4 patients was significantly lower than it was in patients with other T-stages (P<0.001). Pairwise comparisons showed no statistical differences among the T1-T3 patients (P>0.05). The positive rate of Glut-1 and the relative expression of Glut-1 miRNA increased with the rise in T stages, and there were statistical differences (P<0.001). The comparisons among patients at different N and M stages demon-

Table 3. Comparison of the Glut-1 expressions in the HCC tissues of patients at different ages

Group	Number of patients	Relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
≥60 ages	42	0.42±0.17	7 (16.67)	35 (83.33)	1.93±0.16
<60 ages	222	0.41±0.32	31 (13.96)	191 (86.04)	1.93±0.21
F/t	-	0.123	0.209		0.099
P	-	0.876	0.647		0.921

Table 4. Comparison of the Glut-1 expressions in the HCC tissues of patients of different genders

Group	Number of patients	Relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
Male	219	0.41±0.32	30 (13.70)	189 (86.30)	1.93±0.20
Female	45	0.40±0.34	8 (17.78)	37 (82.22)	1.93±0.18
F/t	-	0.283	0.504		0.087
P	-	0.804	0.478		0.931

Table 5. Comparison of the Glut-1 expressions in tumors of different sizes

Group	Number of patients	relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
≤2 cm	22	0.76±0.18	12 (54.55)	10 (45.45)	1.74±0.09
2.1-4.9 cm	115	0.66±0.11**	20 (17.39)	95 (82.61)***	1.86±0.13**
≥5.0 m	127	0.25±0.20$^{***,\#\#\#}$	6 (4.72)	121 (95.28)$^{***,\#\#\#}$	2.03±0.22$^{***,\#\#\#}$
F/t	-	63.053	528.000		43.030
P	-	<0.001	<0.001		<0.001

Note: **P<0.01, ***P<0.001, compared with ≤2 cm; $^{\#\#\#}$P<0.001, compared with 2.1-4.9 cm.

Table 6. Comparison of the Glut-1 expressions among patients with varying AFP levels

Group	Number of patients	relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
<40	66	0.71±0.20	20 (30.30)	46 (69.70)	1.81±0.12
40-399	100	0.62±0.13	14 (14.00)	86 (86.00)*	1.86±0.13*
≥400	98	0.22±0.20$^{***,\#\#\#}$	4 (4.08)	94 (95.92)$^{***,\#}$	2.08±0.20$^{***,\#\#\#}$
F/t	-	65.232	528.000		43.030
P	-	<0.001	<0.001		<0.001

Note: *P<0.05, ***P<0.001, compared with <40; $^{\#}$P<0.05, $^{\#\#\#}$P<0.001, compared with 40-399.

strated that the relative expression levels of lincRNA-p21 in patients at the N1 and M1 stages were lower than those at the N0 and M0 stages, but the relative expression levels of Glut-1 miRNA were higher, and they were significantly different (P<0.001; **Tables 7-9**).

Comparison of the Glut-1 expressions in HCC with the varying degrees of differentiation

The relative expressions of lincRNA-p21 in the poorly-differentiated HCC tissues was lower than it was in the moderately-differentiated and well-differentiated HCC tissues, but the positive rate of Glut-1 and the relative expression of Glut-1 miRNA were higher, and there were significant differences (all P<0.05). The relative expression of lincRNA-p21 in the moderately-differentiated HCC tissues was lower than it was in the well-differentiated HCC tissues, but the positive rate of Glut-1 and the relative expression of Glut-1 miRNA were higher, and they were significantly different (P<0.05; **Table 10**).

Table 7. Comparison of the Glut-1 expressions among patients at different T stages

Group	Number of patients	relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
T1	80	0.44±0.29	19 (23.75)	61 (76.25)	1.79±0.11
T2	47	0.47±0.27	9 (19.15)	38 (80.85)	1.88±0.12***
T3	114	0.43±0.28	10 (8.77)	104 (91.23)**	1.98±0.16***,###
T4	23	0.07±0.05***,###,@@@	0 (0.00)	23 (100.00)***,#	2.20±2.0***,###,@@@
F/t	-	6.055	13.337		
P	-	0.001	0.004		<0.001

Note: **P<0.01, ***P<0.001, compared with T1; #P<0.05, ###P<0.001, compared with T2; @@@P<0.001, compared with T3.

Table 8. Comparison of the Glut-1 expressions in patients at different N stages

Group	Number of patients	relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
N0	248	0.44±0.29	37 (14.92)	211 (85.08)	1.90±0.17
N1	16	0.23±0.22	1 (6.25)	15 (93.75)	2.35±0.18
F/t	-	2.662	0.917		10.285
P	-	0.009	0.338		<0.001

Table 9. Comparison of the Glut-1 expressions in patients at different M stages

Group	Number of patients	relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
M0	235	0.44±0.28	37 (15.72)	198 (84.28)	1.90±0.17
M1	29	0.13±0.16	1 (3.45)	28 (96.55)	2.22±0.21
F/t	-	4.661	3.168		9.650
P	-	0.001	0.075		<0.001

Survival of the Glut-1 positive and negative patients

The median survival time (29 months; 95% CI: 27.659-30.341) of the Glut-1 negative patients was higher than the survival time (25 months; 95% CI: 24.133-25.867) of the Glut-1 positive patients (χ^2=30.950, P<0.001; **Figure 2**).

Correlation between relative expression of lincRNA-p21 and Glut-1

The relative expressions of lincRNA-p21 in the Glut-1 positive patients were lower than they were in Glut-1 negative patients, and the differences were statistically significant (P<0.05; **Table 11**). Results from the correlation analysis revealed that r=-0.256, and P=0.017. The relative expression of lincRNA-p21 was negatively correlated to Glut-1 miRNA.

Discussion

Previous studies have demonstrated that lincRNA-p21 expression is down-regulated in the lung cancer tissues of patients, and there is a correlation between lincRNA-p21 expression and prognosis. LincRNA-p21 may be a marker of prognosis for HCC patients [26]. The expression of lincRNA-p21 in diffuse large B-cell lymphoma tissues is significantly down-regulated compared to the expression in normal tissues [27]. In prostate cancer, lincRNA-p21 expression is down-regulated, and a lower expression of lincRNA-p21 is associated with lower survival rates [28]. Evidence shows that lincRNA-p21 inhibits the growth and invasion of HCC cells, and decreased lincRNA-p21 expression is associated with higher stages, grades and the vascular invasion of HCC [29]. The expression of lincRNA-p21 in colorectal cancer tissues is significantly decreased, and it is negatively correlated to the stages and vascular invasion of colorectal cancer [30]. The above studies indicate that the expression of lincRNA-p21 is down-regulated in tumor tissues. Likewise, in the present study, we also found that the relative expression of lincRNA-p21 in HCC tissues was lower than it was in adjacent normal tissues. As the tumor size became larger, the AFP level increased, the T stages were higher, the distant metastasis and differentiation of the lymph nodes were

Table 10. Comparison of the Glut-1 expressions in HCC with varying degrees of differentiation

Group	Number of patients	relative expression of lincRNA-p21	Glut-1 (-) patients	Glut-1 (+) patients	Glut-1 miRNA
Poorly differentiated	70	0.73±0.21	4 (5.71)	66 (94.29)	2.14±0.19
Intermediately differentiated	152	0.61±0.15***	19 (12.50)	133 (87.50)	1.89±0.14***
Highly differentiated	42	0.25±0.19***,###	15 (35.71)	27 (64.29)***,###	1.73±0.08***,###
F/t	-	72.382		20.216	43.030
P	-	<0.001		<0.001	<0.001

Note: ***P<0.001, ###P<0.001, compared with poorly differentiated; ###P<0.001, compared with intermediately differentiated.

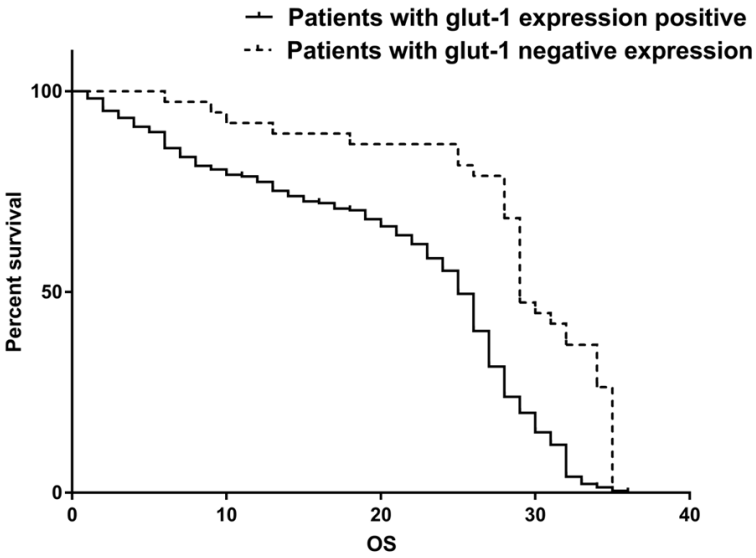

Figure 2. The survival of Glut-1 positive and negative patients.

Table 11. Different expressions of Glut-1 and the relative expressions of lincRNA-p21

Group	Number of patients	Relative expression of lincRNA-p21
Glut-1(-)	38	0.48±0.31
Glut-1(+)	226	0.31±0.21
F/t	-	2.123
P	-	0.014

poorer, and the relative expression of lincRNA-p21 decreased, which is consistent with the findings from the above studies.

A study of Glut-1 from abroad indicates that elevated Glut-1 in oral epithelial dysplasia indicates an increased risk for oral cancer [31]. In a study of endometrial carcinoma, no Glut-1 expression was found in the endometria of healthy women; however, Glut-1 expression was elevated in endometrial carcinoma and atypical hyperplasia tissues, and Glut-1 expression was higher in endometrial carcinoma than in atypical hyperplasia tissues [32]. A prior study involving the roles of Glut-1 in the metastasis and invasion of colorectal cancer showed that Glut-1 was elevated in the cells of stages II and III colorectal cancer compared with the stage I cells, suggesting that Glut-1 may be an important marker for colorectal cancer staging [33]. Another study of colorectal cancer found that the level of Glut-1 was associated with age, tumor stages, and lymphatic metastasis in patients with colorectal cancer [34]. This means that even in a hypoxic environment with an elevated Glut-1 expression, the proliferation and metastasis of tumors and the invasion of surrounding tissues is also induced [35]. In the present study, we found that the larger the size of the tumors was, the higher the AFP value was. Also, the higher the T stage was, and the poorer the distant metastasis and differentiation of the lymph nodes were, the higher the expression of Glut-1 was, indicating that elevated Glut-1 expression is correlated with a stronger tumor invasive capacity, which is consistent with the above findings.

In a prior study investigating the effect of Glut-1 on the prognosis of patients with tumors, 269 lung cancer patients were enrolled as subjects. The positive rate of Glut-1 in phosphorous cell carcinoma of the lungs was 99%, higher than the rate (50%) in lung adenocarcinoma, and they were significantly different. Further multivariate regression analysis revealed that elevated Glut-1 was negatively correlated with sur-

vival in patients with lung adenocarcinoma [36]. In a study of pancreatic cancer, elevated Glut-1 was found to be associated with decreased overall survival after the resection of pancreatic carcinoma [37]. In another study of gastrointestinal stromal tumors, the elevation of Glut-1 and CD63 led to decreases in overall survival [38]. A meta-analysis on the effect of Glut-1 on tumors indicated elevated Glut-1 was associated with tumor stages, differentiation degree, and tumor size, and reversely correlated with the rates of disease-free survival and overall survival in patients [39]. In the current study, we also found that the survival of Glut-1 positive patients was significantly shorter than the survival of the Glut-1 negative ones, suggesting that elevated Glut-1 increases the invasive capacity of tumors and leads to shorter survival in patients prone to metastasis. The findings conformed to those of the above studies.

In the current study, our research showed that the relative expressions of lincRNA-p21 in Glut-1 positive patients were lower than they were in Glut-1 negative ones. The relative expressions of lincRNA-p21 were negatively correlated with Glut-1 expression. LincRNA-p21 activates the activity of the zinc finger E-box-binding protein, thereby inhibiting glucose uptake [40]. The upregulation of Glut-1 activates the mTOR signaling pathway to promote glycolysis [41]. Multiple studies indicate that expressions of various lncRNAs affect Glut-1 expression, and in turn it has an effect on glucose uptake by tumor cells [42, 43]. The low expression of lincRNA-p21 inhibits P53-mediated glucose metabolism and plays a crucial role in regulating glucose metabolism in the HCC patients. The p53 signaling pathway, a negative regulator of glycolysis, realizes its limitation to glucose uptake by inhibiting the generation of Glut-1 [7, 8]. In the present study, we found that the relative expression of lincRNA-p21 was negatively correlated with Glut-1 expression, which may be correlated to the above mechanisms. The sample size was small in the present study, so we should further expand the sample size to conduct a multi-center study exploring the mechanisms of lincRNA-p21 in HCC.

In conclusion, lincRNA-p21 and Glut-1 play important roles in the presence and progression of HCC. Decreased lincRNA-p21 and elevated Glut-1 suggest a poor prognosis, and they can be used as biomarkers for judging the malignancy and prognosis of HCC.

Acknowledgements

This work was supported by the National Natural Science Foundation of China for The combined effects of Kras mutations and low Pten expression on malignant transformation of biliary tree stem/progenitor cells within peribiliary glands of the intrahepatic large bile ducts and the potential molecular mechanism (81560460/H1602) and the Self-Raised Scientific Research Fund of the Ministry of Health in Guangxi Zhuang Autonomous Region for Effects of parenteral and enteral immune nutrition on nutritional status and immune function of patients after pancreaticoduodenectomy (Z2016479).

Disclosure of conflict of interest

None.

Address correspondence to: Jianhong Zhong and Ningfu Peng, Department of Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, No. 71 Hedi Road, Qingxiu District, Nanning 530021, Guangxi Zhuang Autonomous Region, China. Tel: +86-0771-5330855; Fax: +86-0771-5312000; E-mail: zhongjianhong55jh@163.com (JHZ); pengningfu7pnf7@163.com (NFP)

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.

[2] Moosavy SH, Davoodian P, Nazarnezhad MA, Nejatizaheh A, Eftekhar E and Mahboobi H. Epidemiology, transmission, diagnosis, and outcome of hepatitis C virus infection. Electron Physician 2017; 9: 5646-5656.

[3] Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK and Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int 2017; 11: 317-370.

[4] Wang BG, Lv Z, Ding HX, Fang XX, Wen J, Xu Q and Yuan Y. The association of lncRNA-HULC polymorphisms with hepatocellular cancer risk and prognosis. Gene 2018; 670: 148-154.

[5] Chen S, Liang H, Yang H, Zhou K, Xu L, Liu J, Lai B, Song L, Luo H, Peng J, Liu Z, Xiao Y, Chen W and Tang H. LincRNa-p21: function and mechanism in cancer. Med Oncol 2017; 34: 98.

[6] Sanchez Calle A, Kawamura Y, Yamamoto Y, Takeshita F and Ochiya T. Emerging roles of long non-coding RNA in cancer. Cancer Sci 2018; 109: 2093-2100.
[7] Di Leo L, Vegliante R, Ciccarone F, Salvatori I, Scimeca M, Bonanno E, Sagnotta A, Grazi GL, Aquilano K and Ciriolo MR. Forcing ATGL expression in hepatocarcinoma cells imposes glycolytic rewiring through PPAR-alpha/p300-mediated acetylation of p53. Oncogene 2019; 38: 1860-1875.
[8] Schwartzenberg-Bar-Yoseph F, Armoni M and Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res 2004; 64: 2627-2633.
[9] Zhao M and Zhang Z. Glucose transporter regulation in cancer: a profile and the loops. Crit Rev Eukaryot Gene Expr 2016; 26: 223-238.
[10] Sharen G, Peng Y, Cheng H, Liu Y, Shi Y and Zhao J. Prognostic value of Glut-1 expression in pancreatic cancer: results from 538 patients. Oncotarget 2017; 8: 19760-19767.
[11] Tan Z, Yang C, Zhang X, Zheng P and Shen W. Expression of glucose transporter 1 and prognosis in non-small cell lung cancer: a pooled analysis of 1665 patients. Oncotarget 2017; 8: 60954-60961.
[12] Shang R, Wang J, Sun W, Dai B, Ruan B, Zhang Z, Yang X, Gao Y, Qu S, Lv X, Tao K, Wang L, Dou K and Wang D. RRAD inhibits aerobic glycolysis, invasion, and migration and is associated with poor prognosis in hepatocellular carcinoma. Tumour Biol 2016; 37: 5097-5105.
[13] Ding X, Liu J, Liu T, Ma Z, Wen D and Zhu J. miR-148b inhibits glycolysis in gastric cancer through targeting SLC2A1. Cancer Med 2017; 6: 1301-1310.
[14] Gonzalez-Menendez P, Hevia D, Mayo JC and Sainz RM. The dark side of glucose transporters in prostate cancer: are they a new feature to characterize carcinomas? Int J Cancer 2018; 142: 2414-2424.
[15] Kurahara H, Maemura K, Mataki Y, Sakoda M, Iino S, Kawasaki Y, Arigami T, Mori S, Kijima Y, Ueno S, Shinchi H and Natsugoe S. Significance of glucose transporter type 1 (GLUT-1) expression in the therapeutic strategy for pancreatic ductal adenocarcinoma. Ann Surg Oncol 2018; 25: 1432-1439.
[16] Labak CM, Wang PY, Arora R, Guda MR, Asuthkar S, Tsung AJ and Velpula KK. Glucose transport: meeting the metabolic demands of cancer, and applications in glioblastoma treatment. Am J Cancer Res 2016; 6: 1599-1608.
[17] Yang J, Wen J, Tian T, Lu Z, Wang Y, Wang Z, Wang X and Yang Y. GLUT-1 overexpression as an unfavorable prognostic biomarker in patients with colorectal cancer. Oncotarget 2017; 8: 11788-11796.
[18] Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? Nat Rev Cancer 2016; 16: 635-649.
[19] Hutton JE, Wang X, Zimmerman LJ, Slebos RJ, Trenary IA, Young JD, Li M and Liebler DC. Oncogenic KRAS and BRAF drive metabolic reprogramming in colorectal cancer. Mol Cell Proteomics 2016; 15: 2924-2938.
[20] Zhou JC, Zhang JJ, Zhang W, Ke ZY, Ma LG and Liu M. Expression of GLUT-1 in nasopharyngeal carcinoma and its clinical significance. Eur Rev Med Pharmacol Sci 2017; 21: 4891-4895.
[21] Vaz CV, Marques R, Alves MG, Oliveira PF, Cavaco JE, Maia CJ and Socorro S. Androgens enhance the glycolytic metabolism and lactate export in prostate cancer cells by modulating the expression of GLUT1, GLUT3, PFK, LDH and MCT4 genes. J Cancer Res Clin Oncol 2016; 142: 5-16.
[22] Tang Y, Cao K, Wang Q, Chen J, Liu R, Wang S, Zhou J and Xie H. Silencing of CerS6 increases the invasion and glycolysis of melanoma WM35, WM451 and SK28 cell lines via increased GLUT1-induced downregulation of WNT5A. Oncol Rep 2016; 35: 2907-2915.
[23] Primary liver cancer diagnosis and treatment specification (2017 edition). J Dig Oncol (Electronic Version) 2017; 9: 213-228.
[24] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, Jenkins RB, Press MF, Spears PA, Vance GH, Viale G, McShane LM and Dowsett M. Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of american pathologists clinical practice guideline focused update. J Clin Oncol 2018; 36: 2105-2122.
[25] Fang F, Chang RM, Yu L, Lei X, Xiao S, Yang H and Yang LY. MicroRNA-188-5p suppresses tumor cell proliferation and metastasis by directly targeting FGF5 in hepatocellular carcinoma. J Hepatol 2015; 63: 874-885.
[26] Castellano JJ, Navarro A, Viñolas N, Marrades RM, Moises J, Cordeiro A, Saco A, Muñoz C, Fuster D, Molins L, Ramirez J and Monzo M. LincRNA-p21 impacts prognosis in resected non-small cell lung cancer patients through angiogenesis regulation. J Thorac Oncol 2016; 11: 2173-2182.
[27] Isin M, Ozgur E, Cetin G, Erten N, Aktan M, Gezer U and Dalay N. Investigation of circulating lncRNAs in B-cell neoplasms. Clin Chim Acta 2014; 431: 255-259.
[28] Aird J, Baird AM, Lim MCJ, McDermott R, Finn SP and Gray SG. Carcinogenesis in prostate cancer: the role of long non-coding RNAs. Non-coding RNA Res 2018; 3: 29-38.

[29] Jia M, Jiang L, Wang YD, Huang JZ, Yu M and Xue HZ. lincRNA-p21 inhibits invasion and metastasis of hepatocellular carcinoma through notch signaling-induced epithelial-mesenchymal transition. Hepatol Res 2016; 46: 1137-1144.
[30] Zhai H, Fesler A, Schee K, Fodstad O, Flatmark K and Ju J. Clinical significance of long intergenic noncoding RNA-p21 in colorectal cancer. Clin Colorectal Cancer 2013; 12: 261-266.
[31] Yan S, Coffing BN, Li Z, Xie H, Brennick JB, Beg HA, Froehlich HM and Wells WA. Diagnostic and prognostic value of ProEx C and GLUT1 in melanocytic lesions. Anticancer Res 2016; 36: 2871-2880.
[32] Němejcová K, Rosmusová J, Bártů M, Důra M, Tichá I and Dundr P. Expression of Glut-1 in normal endometrium and endometrial lesions: analysis of 336 cases. Int J Surg Pathol 2017; 25: 389-396.
[33] Chung FY, Huang MY, Yeh CS, Chang HJ, Cheng TL, Yen LC, Wang JY and Lin SR. GLUT1 gene is a potential hypoxic marker in colorectal cancer patients. BMC Cancer 2009; 9: 241.
[34] GabAllah GMK, El-Din Habib MS, Soliman SE, Kasemy ZA and Gohar SF. Validity and clinical impact of glucose transporter 1 expression in colorectal cancer. Saudi J Gastroenterol 2017; 23: 348-356.
[35] Saigusa S, Toiyama Y, Tanaka K, Okugawa Y, Fujikawa H, Matsushita K, Uchida K, Inoue Y and Kusunoki M. Prognostic significance of glucose transporter-1 (GLUT1) gene expression in rectal cancer after preoperative chemoradiotherapy. Surg Today 2012; 42: 460-469.
[36] Koh YW, Lee SJ and Park SY. Differential expression and prognostic significance of GLUT1 according to histologic type of non-small-cell lung cancer and its association with volume-dependent parameters. Lung Cancer 2017; 104: 31-37.
[37] Davis-Yadley AH, Abbott AM, Pimiento JM, Chen DT and Malafa MP. Increased expression of the glucose transporter type 1 gene is associated with worse overall survival in resected pancreatic adenocarcinoma. Pancreas 2016; 45: 974-979.
[38] Lewitowicz P, Matykiewicz J, Koziel D, Chrapek M, Horecka-Lewitowicz A and Gluszek S. CD63 and GLUT-1 overexpression could predict a poor clinical outcome in GIST: a study of 54 cases with follow-up. Gastroenterol Res Pract 2016; 2016: 6478374.
[39] Yu M, Yongzhi H, Chen S, Luo X, Lin Y, Zhou Y, Jin H, Hou B, Deng Y, Tu L and Jian Z. The prognostic value of GLUT1 in cancers: a systematic review and meta-analysis. Oncotarget 2017; 8: 43356-43367.
[40] Song Y, Li X, Zeng Z, Li Q, Gong Z, Liao Q, Li X, Chen P, Xiang B, Zhang W, Xiong F, Zhou Y, Zhou M, Ma J, Li Y, Chen X, Li G and Xiong W. Epstein-Barr virus encoded miR-BART11 promotes inflammation-induced carcinogenesis by targeting FOXP1. Oncotarget 2016; 7: 36783-36799.
[41] Wei S, Fan Q, Yang L, Zhang X, Ma Y, Zong Z, Hua X, Su D, Sun H, Li H and Liu Z. Promotion of glycolysis by HOTAIR through GLUT1 upregulation via mTOR signaling. Oncol Rep 2017; 38: 1902-1908.
[42] Liu X and Gan B. lncRNA NBR2 modulates cancer cell sensitivity to phenformin through GLUT1. Cell Cycle 2016; 15: 3471-3481.
[43] Zou ZW, Ma C, Medoro L, Chen L, Wang B, Gupta R, Liu T, Yang XZ, Chen TT, Wang RZ, Zhang WJ and Li PD. LncRNA ANRIL is up-regulated in nasopharyngeal carcinoma and promotes the cancer progression via increasing proliferation, reprograming cell glucose metabolism and inducing side-population stem-like cancer cells. Oncotarget 2016; 7: 61741-61754.