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BRIEF ARTICLE

A polymorphism within *ErbB4* is associated with risk for hepatocellular carcinoma in Chinese population

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Abstract

AIM: To investigate the association between hepatocellular carcinoma (HCC) susceptibility and a 12-bp insertion/deletion polymorphism (rs6147150) in the 3'UTR of *ErbB4*.

METHODS: Using a case-control design, the rs6147150 genotypes in 270 patients with HCC and 270 healthy controls were determined by direct polymerase chain reaction and polyacrylamide gel electrophoresis. Logistic regression was used to analyze the association between the polymorphism and cancer risk.

RESULTS: Computational modeling suggested that rs6147150 was located in the seed region of hsa-let-7c, a potential target sequence in *ErbB4* 3'UTR. Logistic regression analysis showed that, compared with individuals homozygous for wild-type, heterozygotes [adjusted odds ratio (OR) = 1.48, 95% confidence interval (CI) = 1.03-2.17, P = 0.034] and individuals homozygous for 12-bp del/del (OR = 2.50, 95% CI = 1.37-4.56, P = 0.001) were at significantly higher risk of HCC. Carriers of the "del" allele of rs6147150 had a 1.59-fold increased risk for HCC (95% CI = 1.22-2.07, P = 0.003).

CONCLUSION: rs6147150 may be associated with HCC risk, in part through let-7c-mediated regulation, and may be involved in the pathogenesis of HCC in Chinese populations.

Key words: Hepatocellular carcinoma; *ErbB4*; rs6147150; Insertion/deletion polymorphism

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INTRODUCTION

Hepatocellular carcinoma (HCC) is an epithelial cancer originating from hepatocytes or their progenitor cells and is the fifth most common malignancy worldwide[1]. Carcinogenesis of HCC is a complex, multistep process, associated with various risk factors, including chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, cirrhosis and exposure to carcinogens[2]. Chronic HBV infection is by far the most important risk factor for HCC in China and sub-Saharan Africa, the regions of highest incidence of HCC. Epidemiological studies

have provided evidence that genetic factors are important in determining an individual's susceptibility to HCC. Advances in knowledge of the molecular pathogenesis of HCC have resulted in significant improvements in the therapeutic management of the disease[3]. For example, genetic variants within nuclear factor-κ B (NF-κ B) signaling pathways may be involved in HBV-associated hepatocarcinogenesis[4]. Significant progress in understanding the genetic predisposition to HCC has been provided by genome-wide association studies (GWAS) and intensive international collaboration[5]. However, the genetic basis of susceptibility to HCC is still poorly understood and early detection is limited by the lack of reliable clinical and molecular markers.

miRNAs are a class of endogenous, small (21-23 nucleotide), noncoding but functional RNAs. Mature miRNAs can be generated by sequential processing of primary miRNA transcripts by Drosha and Dicer enzymes, and may act as posttranscriptional regulators of gene expression by complementary base pairing to messenger RNAs[6]. Misregulation of miRNA expression has been linked to many types of cancer[7]. miRNAs recognize their targets mainly through limited base-pairing interactions between the 5' end of the miRNA (i.e., nucleotides 2-8, the seed region) and complementary sequences in the 3' untranslated regions (3'UTRs) of the target mRNAs[6]. The binding of miRNA to mRNA is critical for regulating mRNA level and protein expression. Genetic changes in the 3'UTR targeted by miRNAs have been found to alter the strength of miRNA binding, affecting the regulation of target genes and an individual's risk of cancer[8-10].

The epidermal growth factor (EGF) family of receptor tyrosine kinases consists of four members, ErbB1, ErbB2, ErbB3 and ErbB4[11]. The four ErbB receptors are selectively activated by a number of EGF-like growth factors leading to cellular responses, such as proliferation, differentiation, migration, and/or survival. Aberrant expression or activity of EGFR and ErbB2 have been strongly linked to the etiology of several human epithelial cancers including head and neck squamous cell carcinoma, non-small-cell lung cancer, colorectal cancer, and breast cancer[12]. Activating mutations in ErbB4 have been observed in metastatic melanoma, providing strong evidence for an oncogenic role of ErbB4 in cancer[13]. Furthermore, ErbB4 expression is lower in HCC than in adjacent noncancerous tissues[14]. However, no studies to date have examined the effects of *ErbB4* polymorphisms on susceptibility to HCC. Using *in-silico* analysis, we identified a 12-bp (AAAATAGGATTG) insertion/deletion polymorphism (rs6147150) in the 3'UTR of *ErbB4*, located within the seed region of the hsa-let-7c potential target sequence. Using a case-control design, we assessed whether this rs6147150 polymorphism influences susceptibility to HCC in a Chinese population.

MATERIALS AND METHODS

In-silico analysis of microRNA-binding

The mature human microRNA sequences were obtained from the microRNA database (miRBase) (http://microrna.sanger.ac.uk). A region consisting of rs6147150 plus 15 bp 5' and 3' was used to analyze hybridization of putative microRNAs. The minimum free energy required for hybridization of putative microRNA and polymorphisms was predicted by miRanda software with default parameters[15]. The difference in energies between wild type and variant was computed as ΔΔG and used to assess the impact of the polymorphism on miRNA binding.

Study populations

Peripheral blood samples were obtained from 270 HCC patients newly diagnosed, hospitalized, and treated in our department from 2004 to 2009, and from 270 cancer-free subjects matched by sex and age and selected from a community-wide nutritional survey conducted in the same regions and at the same time as the cancer patients were recruited. All subjects were unrelated ethnic Han Chinese, and none of the 270 HCC patients had received any medical treatment. HCC patients were excluded if they had: (1) primary or secondary biliary cirrhosis or Budd-Chiari syndrome; (2) autoimmune hepatitis or toxic hepatitis; (3) recurrence of HCC; (4) tumors other than HCC; or (5) liver disease due to parasitosis, diabetes, fatty liver, metabolism disorders or severe cardiovascular diseases. HCC diagnosis was confirmed by a pathological examination combined with positive results on magnetic resonance imaging and/or computerized tomography. Tumor stages were determined according to a modified American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) standard. Subjects who smoked more than two cigarettes per day for more than 1 year were classified as smokers; others were defined as non-smokers. Subjects who consumed at least one drink of alcohol per week were considered alcohol drinkers; others were considered non-drinkers. All participants were negative for antibodies to hepatitis C virus, hepatitis D virus and human immunodeficiency virus. The design of the study was approved by the Ethical Committee of Suzhou Municipal Hospital, and all participants provided written informed consent.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood samples using a Chelex method[16]. DNA fragments containing the polymorphism were amplified using the primers 5'-ATTCCAGAGGCCAATTGTA-3' (forward) and 5'-TTTCCTCACCTGTTTACCAC-3' (reverse). Polymerase chain reaction (PCR) reactions were performed in a total volume of 20 μL, containing 2.0 μL 10 × PCR buffer, 1.5 mmol/L $MgCl_2$, 0.25 mmol/L of each dNTP, 0.5 mmol/L of each primer, 100 ng of genomic DNA, and 1.0 U of Taq DNA polymerase. The amplification protocol consisted of an initial denaturation at 94 ℃ for 5 min, followed by 35 cycles of denaturation for 30 s at 94 ℃, annealing for 30 s at 58 ℃, and extension for 30 s at 72 ℃, followed by a final extension at 72 ℃ for 5 min. The PCR products were analyzed by 7% non-denaturing polyacrylamide gel electrophoresis (PAGE) and visual-

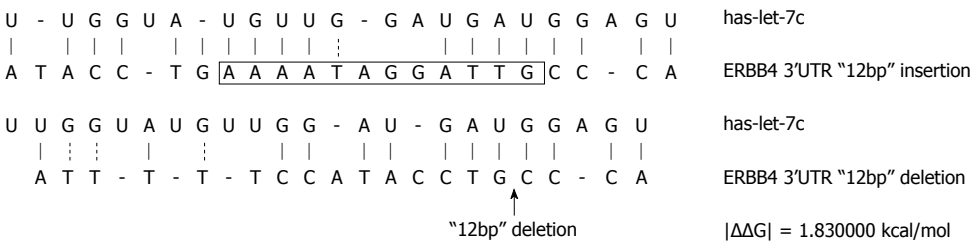

Figure 1 ***In-silico*** **analysis of microRNA-binding.** rs6147150 was located at the fifth nucleotide from the 5' end of has-let-7c. The ΔΔG value between the 12-bp insertion (up) and the 12-bp deletion (down) alleles was calculated to be 1.830000 kcal/mol.

Table 1 Demographic characteristics of the hepatocellular carcinoma case and control groups

Characteristics	Case		Control		*P* value
	n = 270	Frequency (%)	*n* = 270	Frequency (%)	
Age, (yr) (mean ± SD)	50.3 ± 9.2		50.7 ± 9.4		0.91[a]
Gender					
Male	187	69.3	190	70.4	0.78[b]
Female	83	30.7	80	29.6	
Smoking status					
Smoker	79	29.3	85	31.5	0.57[b]
Non-smoker	191	70.7	185	68.5	
Drinking status					
Drinker	120	44.4	116	43.0	0.73[b]
Non-drinker	150	55.6	154	57.0	
Tumor stages					
Ⅰa + Ⅰb	193	71.5			
Ⅱa + Ⅱb	58	21.5			
Ⅲa + Ⅲb	19	7.0			
HBsAg, *n* (%)					
Positive	201	74.4	26	9.6	< 0.0001[b]
Negative	69	25.6	244	90.4	

[a]Two-sided two-sample *t*-tests. [b]χ^2 tests. HBsAg: Hepatitis B surface antigen.

ized by silver staining[17]. The 12-bp deletion allele of rs6147150 yielded a band of 181 bp and the insertion allele yielded a band of 193 bp. To validate the genotyping method, we analyzed 20 randomly selected DNA samples by both direct sequencing and the PCR method; the concurrence rate of these two methods was 100%, indicating that the PCR method is reliable. Genotyping was performed without knowledge of case or control status. Moreover, 10% of the samples were randomly selected for testing by two different persons, with a reproducibility of 100%.

Statistical analysis

Hardy-Weinberg equilibrium was assessed using a goodness-of-fit χ^2 test for biallelic markers. The adjusted odds ratios (ORs) with their 95% confidence intervals (CIs) of the association between polymorphism and HCC risk were estimated by multiple logistic regression models after controlling for sex, age, smoking status, drinking status, tumor stage and HBV infection. In all cases, homozygosity for the most common allele (i.e., ins/ins) was used as the reference category. A binary logistic regression model was used for stratification by HBV infection status. All statistical analyses were performed using Statistic Analysis System software (version 8.0, SAS Institute), with statistical significance defined as $P < 0.05$ and statistical power calculated using PS software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize).

Table 2 Genotype and allele frequencies of rs6147150 among cases and controls, and risk of hepatocellular carcinoma *n* (%)

Genotype/allele	Cases		Controls		OR (95% CI)[1]	*P* value
12N ins/ins	109	40.4	143	53.0	1.00 (Reference)	-
12N ins/del	117	43.3	104	38.5	1.48 (1.03-2.17)	0.034
12N del/del	44	16.3	23	8.5	2.50 (1.37-4.56)	0.001
P_{trend}						0.0006
12N ins	335	62.0	390	72.2	1.00 (Reference)	-
12N del	205	38.0	150	27.8	1.59 (1.22-2.07)	0.0003

[1]adjusted for sex, age, smoking status, drinking status, HBV infection and tumor stage. HBV: Hepatitis B virus; OR: Odds ratio; CI: Confidence interval.

RESULTS

In-silico analysis of microRNA-binding

Computational modeling of the rs6147150 polymorphism suggested that it was located within the hsa-let-7c potential target sequence of the *ErbB4* 3'UTR (Figure 1). The ΔΔG value was calculated as 1.83 kcal/mol.

Association of HCC with the rs6147150 polymorphism

The demographic characteristics of the 270 HCC patients and 270 controls are summarized in Table 1. There were no statistically significant differences in sex distribution, age, or smoking or drinking status between the two groups. As expected, HBV infection was a significant risk factor for HCC. About 74.4% of the cases and 9.6% of the controls were HBsAg-positive ($P < 0.0001$). Genotype distributions showed no deviation from Hardy-Weinberg equilibrium in either cases ($P = 0.149$) or controls ($P = 0.416$). We found that rs6147150 was significantly associated with HCC susceptibility at both the allele and genotype levels (Table 2). Comparing with individuals homozygous for 12N ins/ins, those heterozygous for 12N ins/del (adjusted OR = 1.48, 95% CI = 1.03-2.17, $P = 0.034$) and homozygous for 12N del/del (OR = 2.50, 95% CI = 1.37-4.56, $P = 0.001$) were at significantly in-

Table 3 Stratification analysis based on hepatitis B virus infection status in cases and controls

Genotype	HBV positive					HBV negative				
	Case	%	Control	%	OR[1] (95% CI)	Case	%	Control	%	OR[1] (95% CI)
12N ins/ins	82	40.8	14	53.8	1.00 (Reference)	28	40.6	127	52.0	1.00 (Reference)
12N ins/del	88	43.8	10	38.5	1.51(0.57-3.91)	30	43.5	90	36.9	1.52 (0.79-2.94)
12N del/del	31	15.4	2	7.7	2.64 (0.51-17.59)	11	15.9	27	11.1	1.87 (0.74-4.54)
P_{trend}					P = 0.156					P = 0.083

[1]Adjusted for sex, age, smoking status, drinking status and tumor stage. HBV: Hepatitis B virus; OR: Odds ratio; CI: Confidence interval.

creased risk of HCC after controlling for other covariates (Table 2). We also found that the frequency of the 12-bp deletion or insertion allele differed significantly between the HCC and control groups. The presence of the 12-bp deletion allele was associated with a significantly increased risk of developing HCC (OR = 1.59, 95% CI 1.22-2.07, P = 0.003). HBV stratification analysis showed no significant difference in allele frequency between HBV-positive and HBV-negative groups (Table 3). Using PS software, we estimated a power of 0.94 with an α set at 0.05 to obtain an OR of 2.0.

DISCUSSION

In addition to environmental factors, such as viral infection, an increasing number of novel genetic components identified by GWAS have been found to predispose individuals to HCC. Thus, assessments of functional variants are necessary to determine risks of developing HCC. To our knowledge, this study is the first to evaluate the association between genetic variants in *ErbB4* and HCC susceptibility. Our results indicate that rs6147150 is associated with HCC susceptibility in a Chinese population, possibly through let-7c mediated regulation.

Altered ErbB signaling has been frequently observed during malignant transformation, with ErbB overactivity often implicated in the pathogenesis of several epithelial malignancies[18,19]. In contrast, growth factor receptors with tyrosine kinase activity are known to contribute greatly to the regulation of cell behavior, such as cell growth, proliferation and mortality. ErbB4 is frequently expressed in tumors[20-22]; although, in contrast to EGFR and ErbB2, its role as a tumor-driving oncogene is unclear[23]. Although there is little evidence of an association between ErbB4 and HCC, miRNAs have been shown to modulate ErbB receptor expression and downstream signaling activity, stimulating intense interest in the development of miRNAs as therapeutic molecules and clinical biomarkers in cancer[24]. Because HCC is an epithelial cancer originating from hepatocytes or their progenitors, genetic polymorphisms in *ErbB4* may be associated with susceptibility to HCC.

Since bioinformatics analysis suggests that rs6147150 lies within a predicted binding site (seed region) for let-7c, we hypothesized that let-7c would bind tightly to ErbB4 mRNA transcripts containing the 12-bp deletion allele, negatively regulating ErbB4 expression. Conversely, binding with mRNA transcripts containing the 12-bp insertion allele would be disrupted, resulting in increased ErbB4 expression. An ErbB4-specific ligand, heparin-binding EGF-like growth factor (HB-EGF), has been shown to be involved in the development and/or progression of human HCC in an autocrine and/or a paracrine manner, especially during the early stages of HCC[25,26]. Therefore, aberrant expression of ErbB4 would influence the specific binding of HB-EGF and increase the risk for HCC. miRNAs of the let-7 family are highly conserved in bilateral animals and control stem cell division and differentiation[27]. They also function as tumor suppressors and inhibit cell proliferation and tumorigenesis. Low levels of expression of let-7 have been observed in a variety of cancers, including HCC[28].

Taken together, our results suggest that common genetic polymorphisms in *ErbB4* may influence HCC risk, at least in part by let-7c-mediated regulation which may be involved in the pathogenesis of HCC. Our results may provide a greater understanding of the mechanisms of hepatocarcinogenesis and may help in the identification of diagnostic markers for HCC. However, it is important to determine whether the association between this polymorphism and HCC risk also applies to other populations. Finally, additional functional studies are required to fully understand the involvement of *ErbB4* polymorphisms in predisposition to HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, with an increasing incidence worldwide. Epidemiological studies have indicated that genetic factors are important in determining an individual's susceptibility to HCC. However, the genetic basis of susceptibility to HCC is still poorly understood and early detection of HCC is infrequent because of the lack of reliable markers. Studies focusing on functional variants in these findings are therefore indispensable.

Research frontiers

Recent genome-wide association studies, which are routinely used to identify common polymorphisms that underlie disease susceptibility in a large population, have enhanced our understanding of the genetic predisposition to HCC. These findings have led to the identification of several genetic variants that may modulate the risk of HCC.

Innovations and breakthroughs

Relative to individuals homozygous for the rs6147150 12-bp ins/ins allele, homozygotes for 12-bp del/del and heterozygotes are at significantly higher risk of HCC. Carriers of the "del" allele are associated with a 1.59-fold increased risk of HCC relative to non-carriers. These findings suggest that common genetic polymorphisms in *ErbB4* may influence HCC risk, at least in part *via* let-

7c-mediated regulation, which may be involved in the pathogenesis of HCC in Chinese populations.

Applications

This preliminary report may help clarify the molecular pathogenesis of hepatocarcinogenesis. Polymorphisms within *ErbB4* may be used as potential markers for HCC predisposition in Chinese populations.

Peer review

This study shows that rs6147150 alleles are significantly associated with the risk of HCC, at least in part through let-7c-mediated regulation, which may be involved in the pathogenesis of HCC. The study design is reasonable and sample size is acceptable.

REFERENCES

1 **Caldwell S**, Park SH. The epidemiology of hepatocellular cancer: from the perspectives of public health problem to tumor biology. *J Gastroenterol* 2009; **44** Suppl 19: 96-101
2 **Gomaa AI**, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. *World J Gastroenterol* 2008; **14**: 4300-4308
3 **Whittaker S**, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene* 2010; **29**: 4989-5005
4 **He Y**, Zhang H, Yin J, Xie J, Tan X, Liu S, Zhang Q, Li C, Zhao J, Wang H, Cao G. IkappaBalpha gene promoter polymorphisms are associated with hepatocarcinogenesis in patients infected with hepatitis B virus genotype C. *Carcinogenesis* 2009; **30**: 1916-1922
5 **Zhang H**, Zhai Y, Hu Z, Wu C, Qian J, Jia W, Ma F, Huang W, Yu L, Yue W, Wang Z, Li P, Zhang Y, Liang R, Wei Z, Cui Y, Xie W, Cai M, Yu X, Yuan Y, Xia X, Zhang X, Yang H, Qiu W, Yang J, Gong F, Chen M, Shen H, Lin D, Zeng YX, He F, Zhou G. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. *Nat Genet* 2010; **42**: 755-758
6 **Pillai RS**. MicroRNA function: multiple mechanisms for a tiny RNA? *RNA* 2005; **11**: 1753-1761
7 **Wiemer EA**. The role of microRNAs in cancer: no small matter. *Eur J Cancer* 2007; **43**: 1529-1544
8 **Chen K**, Song F, Calin GA, Wei Q, Hao X, Zhang W. Polymorphisms in microRNA targets: a gold mine for molecular epidemiology. *Carcinogenesis* 2008; **29**: 1306-1311
9 **Nicoloso MS**, Sun H, Spizzo R, Kim H, Wickramasinghe P, Shimizu M, Wojcik SE, Ferdin J, Kunej T, Xiao L, Manoukian S, Secreto G, Ravagnani F, Wang X, Radice P, Croce CM, Davuluri RV, Calin GA. Single-nucleotide polymorphisms inside microRNA target sites influence tumor susceptibility. *Cancer Res* 2010; **70**: 2789-2798
10 **Landi D**, Gemignani F, Barale R, Landi S. A catalog of polymorphisms falling in microRNA-binding regions of cancer genes. *DNA Cell Biol* 2008; **27**: 35-43
11 **Linggi B**, Carpenter G. ErbB receptors: new insights on mechanisms and biology. *Trends Cell Biol* 2006; **16**: 649-656
12 **Kruser TJ**, Wheeler DL. Mechanisms of resistance to HER family targeting antibodies. *Exp Cell Res* 2010; **316**: 1083-1100
13 **Prickett TD**, Agrawal NS, Wei X, Yates KE, Lin JC, Wunderlich JR, Cronin JC, Cruz P, Rosenberg SA, Samuels Y. Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in ERBB4. *Nat Genet* 2009; **41**: 1127-1132
14 **Ito Y**, Takeda T, Sakon M, Tsujimoto M, Higashiyama S, Noda K, Miyoshi E, Monden M, Matsuura N. Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. *Br J Cancer* 2001; **84**: 1377-1383
15 **John B**, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. *PLoS Biol* 2004; **2**: e363
16 **Walsh PS**, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991; **10**: 506-513
17 **Allen RC**, Graves G, Budowle B. Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. *Biotechniques* 1989; **7**: 736-744
18 **Hollmén M**, Elenius K. Potential of ErbB4 antibodies for cancer therapy. *Future Oncol* 2010; **6**: 37-53
19 **Hynes NE**, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* 2005; **5**: 341-354
20 **Junttila TT**, Sundvall M, Lundin M, Lundin J, Tanner M, Härkönen P, Joensuu H, Isola J, Elenius K. Cleavable ErbB4 isoform in estrogen receptor-regulated growth of breast cancer cells. *Cancer Res* 2005; **65**: 1384-1393
21 **Ljuslinder I**, Malmer B, Isaksson-Mettävainio M, Oberg A, Henriksson R, Stenling R, Palmqvist R. ErbB 1-4 expression alterations in primary colorectal cancers and their corresponding metastases. *Anticancer Res* 2009; **29**: 1489-1494
22 **Rickman OB**, Vohra PK, Sanyal B, Vrana JA, Aubry MC, Wigle DA, Thomas CF. Analysis of ErbB receptors in pulmonary carcinoid tumors. *Clin Cancer Res* 2009; **15**: 3315-3324
23 **Gullick WJ**. c-erbB-4/HER4: friend or foe? *J Pathol* 2003; **200**: 279-281
24 **Barker A**, Giles KM, Epis MR, Zhang PM, Kalinowski F, Leedman PJ. Regulation of ErbB receptor signalling in cancer cells by microRNA. *Curr Opin Pharmacol* 2010; **10**: 655-661
25 **Inui Y**, Higashiyama S, Kawata S, Tamura S, Miyagawa J, Taniguchi N, Matsuzawa Y. Expression of heparin-binding epidermal growth factor in human hepatocellular carcinoma. *Gastroenterology* 1994; **107**: 1799-1804
26 **Ito Y**, Takeda T, Higashiyama S, Sakon M, Wakasa KI, Tsujimoto M, Monden M, Matsuura N. Expression of heparin binding epidermal growth factor-like growth factor in hepatocellular carcinoma: an immunohistochemical study. *Oncol Rep* 2001; **8**: 903-907
27 **Büssing I**, Slack FJ, Grosshans H. let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med* 2008; **14**: 400-409
28 **Gramantieri L**, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, Calin GA, Giovannini C, Ferrazzi E, Grazi GL, Croce CM, Bolondi L, Negrini M. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007; **67**: 6092-6099

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