RESEARCH ARTICLE

# Talin-1 Correlates with Reduced Invasion and Migration in Human Hepatocellular Carcinoma Cells

**Kun-Peng Fang, Jian-Lin Zhang, Yan-Hong Ren, Ye-Ben Qian***

## Abstract

**Background: Talin-1 is a cytoskeleton protein that participates in cell migration and plays a role in tumor formation, migration, and metastasis in different types of cancer. Chinese investigators have observed that the levels of Talin-1 protein and mRNA expression in HCC tissues are significantly lower than in the adjacent non-cancerous tissue. However, Japanese investigators have reported that Talin-1 is upregulated in HCC. Tln2 as homologous gene of Tln-1, which encodes a very similar protein, but the role of Talin-2 is very little known in primary liver cancer (PLC). We investigated whether the expression of Talin-1 in PLC may be associated with the histological subtype as well as the role of Talin-1 in tumor cell invasion and migration using human hepatocellular carcinoma cell lines. Materials and Methods: We measured the mRNA expression levels of Talin-1 and Talin-2 in five human liver cancer cell lines and normal human liver cell ($LO_2$ cell line) by real-time PCR and the protein expression levels of Talin-1 by Western blot. Migration and invasion of the cells were assessed using transwell assays and cell scratch experiments, respectively, and proliferation was assessed by soft AGAR colony formation. Results: Talin-1 and Talin-2 expression differed significantly between the five human liver cancer cell lines and $LO_2$ cell line ($p<0.05$). Compared with the $LO_2$ cell line, the invasion and migration capabilities of the five cancer cell lines differed significantly ($p<0.05$). Similarly, the colony-forming ability differed ($p<0.05$). Conclusions: High levels of Talin-1 expression are correlated with reduced invasion and migration as well as decreased malignancy in human liver cancer cell lines; the suppression of Talin-1 promotes invasion and migration. In addition, Talin-2 may be correlated with invasion and migration in human hepatocellular carcinoma.**

**Keywords:** Hepatocellular carcinoma - HCC - Talin-1 - Talin-2 - migration - invasion - cell lines

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## Introduction

Primary liver cancer is the fifth most common cancer worldwide (Schafer et al., 1999; Beer et al., 2002;Kirk et al., 2006; Farazi et al., 2006; Laurent et al., 2006; Zender et al., 2006; Hui, 2009). Hepatocellular carcinoma (HCC) accounts for approximately 85% to 90% of primary liver cancers (Hui, 2009) and is the third leading cause of cancer-related death overall, accounting for more than half a million deaths annually (Bosch et al., 2005; Farazi et al., 2006; El-Serag et al., 2007; Hui, 2009; Zhang et al., 2011), particularly in Southeast Asia and sub-Saharan Africa (El-Serag et al., 2007; Hui, 2009). The highest liver cancer rate in the world is in China (Zhang et al., 2011; Zeinab et al., 2012) accounts for more than 50% of the world's HCC cases (age-standardized incidence rate: men, 35.2/100000; women, 13.3/100000) (Bosch et al., 2005; El-Serag et al., 2007; Hui, 2009; Zhang et al., 2011). HCC, like other cancers, is characterized by a multistage process of tumor progression (Takayama et al., 1990; Kirk et al., 2006; Kanamori et al., 2011), including invasion of the surrounding tissues, migration, and the colonization of distant sites in the body. However, the changes occurring during the malignant transformation of HCC are still not well characterized (Takayama et al., 1990; Takayama et al., 1998; International Consensus Group for Hepatocellular Neoplasia., 2009; Kanamori et al., 2011). Several studies have reported that tumor differentiation is predictive of prognosis and recurrence (Lauwers et al., 2002; Roayaie et al., 2004; Zavaglia et al., 2005; Shafizadeh 2013).

There are two Talin genes in vertebrates, Tln1 and Tln2, which encode very similar proteins (74% amino acid sequence identity) (Senetar, 2005; Debrand et al., 2009; Monkley et al., 2011; Praekelt et al., 2012). Tln2 appears to be the ancestral gene, with Tln1 arising by gene duplication early in the chordate lineage (Senetar 2005; Senetar et al., 2007; Praekelt et al., 2012).

Talin-1 as a cytoskeletal protein with a molecular mass of 270 kDa has been shown to play a pivotal role in regulating the activity of the integrin family of cell adhesion proteins, coupling integrins to F-actin (Critchley, 2004; Kanamori et al., 2011), and as a focal adhesion protein that binds to multiple adhesion molecules, including integrins, vinculin, focal adhesion kinase (FAK),

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and actin. Moreover, Talin-1 plays an essential role in integrin activation (Giancotti, 1999; Sakamoto et al., 2010). Upon activation, integrins increase the number of functional interactions between a cell and the ECM, thus serving as bidirectional transducers of extracellular and intracellular signals and ultimately regulating adhesion, proliferation, anoikis, survival, and tumor progression (Giancotti, 1999; Fornaro et al., 2001; Calderwood 2004; Sakamoto et al., 2010). But the role and function of Talin-2 as homologous gene of Tln-1 is known very little.

Chinese investigators observed that the levels of Talin-1 protein and mRNA expression in HCC tissues were significantly lower than in the adjacent non-cancerous tissue. However, Japanese investigators have reported that Talin-1 is upregulated in HCC. Egyptian investigators have reported that Serum levels of TLN1 in hepatocellular carcinoma patients were significantly higher.

We investigated whether the expression of Talin-1 in HCC may be associated with the histological subtype of HCC, Thus, we sought to determine the levels of Talin-1 expression in human liver cancer cell lines, to observe the relationship between Talin-1 expression and the invasion and migration of liver cancer cell lines, and to determine the relationship between Talin-1 expression and liver cancer cell colonization.

## Materials and Methods

*Cell culture*

The highly metastatic human liver cancer cell line MHCC-97H and the minimally metastatic liver cancer cell lines MHCC-97L were purchased from Fudan University at the Shanghai Huashan Hospital GanDanYi experiment center. The SMMC-7721, BEL-7402, and HePG-2 cell lines were obtained from the First Affiliated Hospital of Anhui Medical University Central Laboratory. The human normal liver cell line $LO_2$ was obtained from the Beijing Military Laboratory of the College of Life Science. The cells were cultured in DMEM (Invitrogen) containing 10% fetal bovine serum (Invitrogen) and antibiotics (penicillin G/Streptomycin, 50 μg/mL), and the cell culture reagents were purchased from Sangon Biotech (Shanghai) Co., Ltd.

*RNA extraction and real time-PCR*

Total cellular RNA was extracted using the RNeasy Miniprep Kit (Sangon Biotech (Shanghai) Co., Ltd.) according to the manufacturer's instructions. RNA concentrations were quantified at 260/280 nm and treated accordingly with Turbo DNase (Ambion). DNase-treated total RNA (1 $\mu$g) was reverse transcribed with Superscript III reverse transcriptase (Invitrogen) and 500 ng of random primers (promega) in a total volume of 40 $\mu$l as recommended by Invitrogen. In addition, non-reverse-transcribed controls were generated under the same conditions by replacing the Superscript III enzyme with water. The TLN-1 and TLN-2 primer pairs from http://primerdepot.nci.nih/gov were as follows: TLN-1: 5'-3' primer sequence (exon 33,) 5-TCTCCCAAAATGCCAAGAAC-3; 3'-5' primer sequence (exon 34),5-TGGCTATTGGGGTCAGAGAC-3. TLN-2: 5'-3' primer sequence (exon 54,) 5- CTGAGGC TCTTTTCACAGCA -3; 3'-5' primer sequence (exon 55), 5- CTCATCTCATCTGCCAAGCA -3. Amplification was performed under standard conditions at an annealing temperature of 60°C using primers at 600 nM and PCR Ready mix (ThermoScientific) in a volume of 15 $\mu$l. The expression of TLN1 mRNA was quantified using real-time PCR in a Roche Light-Cycler with SybrGreen Mix (Fermentas) and the specific primer sets described above. The primers were used at a final concentration of 300 nM in a 25-$\mu$l reaction volume. One microliter of random-primed cDNA was added to each reaction, and each cDNA sample was amplified in triplicate. The absolute quantity of cDNA in each sample was interpolated on a standard curve obtained from a serial dilution of human foreskin fibroblast (hFF) cDNA. GAPDH was used as an internal normalization control.

*Western blotting*

The cells were lysed in Laemmli buffer, and the proteins were resolved by SDS-PAGE and blotted to PVDF membranes. The following isoform-specific Talin-1 monoclonal antibodies were generated during the course of this study and will be described in more detail elsewhere: anti-Talin-1 97H6 (Abcam).

*Transwell migration assay*

Logarithmic growth phase cells were subjected to 0.25% trypsin digestion, and centrifugal precipitation was used to pellet the cells. The cells were resuspended in DMEM were diluted after adjusting the living cell count to a density of $5\times10^5$ cells/ml. Approximately 200 $\mu$l of the cell suspension was added to each upper chamber of a 24-well plate (BD), and DMEM containing 10% fetal bovine serum broth was placed in the lower chamber. The plate was incubated in a 37°C incubator at 5% $CO_2$ for 24 h. Subsequently, the cells were washed with PBS, and swabs were used to wipe the surfaces of the Transwell membranes. The cells were fixed in 4% paraformaldehyde for 30 min and dyed using crystal violet dye for 25 min. At 200× magnification, the cells were counted in five different fields, and the mean number of cells per field was taken to represent the invasive tumor cells. Indirect detection of cell invasion and migration was determined using the MTT method, and the experiment was repeated three times.

*Scratch test*

Logarithmic growth phase cells were digested in 0.25% trypsin, and the cell pellets were collected by centrifugation. The cells were resuspended in complete medium, and the cell density was adjusted to $1\times10^6$ cells/ml. Three milliliters of cell suspension was added to each well of a 6-well plate, and the cells were subjected to normoxic or hypoxic conditions for 24 h. When the cells reached 80% confluence, the supernatant was removed, and a scratch was made in the middle of the cells equidistant from the edges of the dish. The cells were washed in PBS, and the DMEM was replaced. Once every 6 h of observation, the distance between the cell fronts on either side of the scratch was measured and recorded.

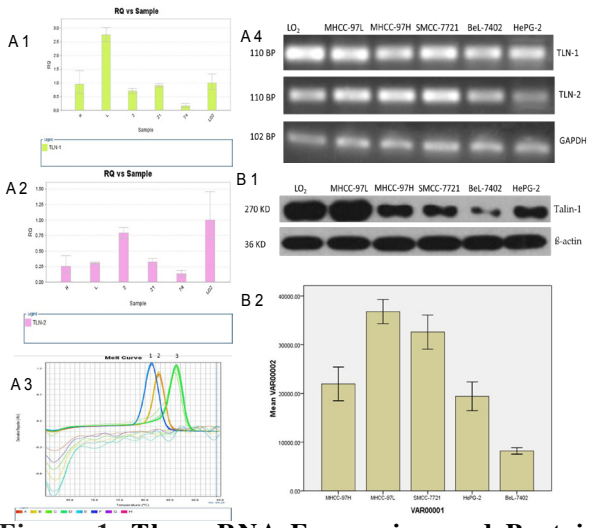


**Figure 1. The mRNA Expression and Protein Expression of Talin-1 and the mRNA Expression of Talin-2 in Five Different Liver Cancer Cell Lines and $LO_2$ Cells**

*Soft agarose colony-forming experiment*

Logarithmic growth phase cells were digested in 0.25% trypsin, and the cell pellets were collected by centrifugation. The cells were resuspended in DMEM and diluted after adjusting the living cell count to a density of $1\times10^3$/ml. Approximately 0.5 ml of the cell suspension (500 cells) was added to culture medium to a volume of 9.4 ml. The cells were incubated at 37°C. To prepare the soft agar, 5% agar was placed in a boiling water bath until it was completely melted. Approximately 1 ml was removed and placed into a sterile small beaker and cooled to 50 degrees. Quickly, 9 ml of complete medium at a temperature of 37°C was added and immediately mixed, and the solution was added to 24-well plates and allowed to solidify at room temperature. The upper agar was prepared by adding the 9.4-ml cell suspension (0.6 ml of 50°C) to 5% agar, mixing quickly, and immediately pouring the solution into the wells of the 24-well plate. The agar was allowed to solidify at room temperature. Each well of the plate contained 40 cells, and three replicates were performed per experimental group. The plates were maintained for 2-3 weeks. Subsequently, the number of clones per plate was assessed using microscopy. Colonies were counted if they contained 50 or more cells. The clone formation rate was calculated, and images were taken. Colony count=total number of colonies /n, clone formation rate=100% (colony number/ infected cells)

*Statistical analysis*

The experimental data were analyzed using SPSS 17.0 software and Microsoft Office Excel 2010. The data are reported as the means±standard deviation, and the groups were compared using one-way analysis of variance. $p<0.05$ indicated statistically significant differences

## Results

*The expression of Talin-1 in liver cancer cell lines and normal liver cell lines*

The mRNA expression of Talin-1 in liver cancer cell lines and normal liver cell lines: Talin-1 mRNA expression differed significantly between liver cancer cell lines and the normal liver cell line ($LO_2$ cell line) ($p<0.0001$). The MHCC–97L cell line exhibited higher Talin-1 expression than $LO_2$ cell line ($p<0.0001$). The Bel-7402 and HePG-2 cell lines exhibited lower Talin-1 mRNA levels than $LO_2$ cell line ($p<0.0001$). The MHCC-97H and SMMC-7721 cell lines also exhibited lower Talin-1 expression than $LO_2$ cell line ($p>0.05$), as shown in Figure 1 (A 1, A 3, A 4) and Table 1.

**Table 1. Talin-1 mRNA Expression in the Five Different Liver Cancer Cell Lines and the LO2 Cell Line**

| Group | n | ΔCt | *p* |
|---|---|---|---|
| MHCC-97H | 6 | 5.5442±0.0106 | [a]0.478 |
| MHCC-97L | 6 | 4.0129±0.0390 | [b]0.000 |
| BEL-7402 | 6 | 8.0109±0.0624 | [b]0.000 |
| SMMC-7721 | 6 | 5.615±0.0095 | [a]0.078 |
| HePG-2 | 6 | 5.9713±0.0562 | [b]0.000 |
| LO2 | 6 | 5.4744±0.0631 | --- |

*The mean difference is significant at the 0.05 level. The five cancer cell lines were compared with $LO_2$, The data are expressed as the means±SD. The experimental data were analyzed by one-way analysis of variance. [a]$p>0.05$ *vs* $LO_2$, [b]$p<0.05$ *vs* $LO_2$

**Table 2. Talin-2 mRNA Expression in the Five Different Liver Cancer Cell Lines and the $LO_2$ Cell Line**

| Group | n | ΔCt | *p* |
|---|---|---|---|
| MHCC-97H | 6 | 14.3348±0.1970 | [b]0.000 |
| MHCC-97L | 6 | 15.4440±0.0212 | [b]0.000 |
| BEL-7402 | 6 | 12.7898±0.1612 | [b]0.000 |
| SMMC-7721 | 6 | 15.4702±0.0301 | [b]0.000 |
| HePG-2 | 6 | 13.3799±0.0173 | [b]0.000 |
| $LO_2$ | 6 | 13.9169±0.1194 | --- |

*The mean difference is significant at the 0.05 level. The five cancer cell lines were compared with $LO_2$, The data are expressed as the means±SD. The experimental data were analyzed by one-way analysis of variance. [b]$p<0.05$ *vs* $LO_2$

*The mRNA expression of Talin-2 in liver cancer cell lines and normal liver cell lines*

Talin-2 mRNA expression differed significantly between liver cancer cell lines and $LO_2$ cell line ($p<0.0001$). the mRNA expression of Talin-2 in five liver cancer cell lines exhibited lower expression than $LO_2$ cell line ($p<0.0001$), as shown in Figure 1 (A 2, A 3, A 4,) and Table 2.

*The protein expression of Talin-1 in liver cancer cells and normal liver cells*

The protein expression of Talin-1 in the liver cancer cell lines and the normal liver cell line differed significantly. MHCC-97L cell line exhibited elevated Talin-1 protein levels ($p<0.05$). The results of the protein analysis paralleled those of the gene expression analysis, as shown in Figure 1 (B 1, B 2).

*The relationship between Talin-1 expression and HCC invasion and migration*

The microscopy images shown in Figure 2 (C) demonstrate that the invasion and migration abilities of the five cancer cell lines differed significantly ($p<0.05$). Compared with the $LO_2$ cell line, the high Talin-1 expressing MHCC-97L cell line exhibited reduced

**Table 3. The Five Different Liver Cancer Cell Lines are Compared with the $LO_2$ Cell Line with Respect to the Ultraviolet Absorption values**

| Group | n | UV (mean±SD) | *p* |
|---|---|---|---|
| MHCC-97H | 6 | 0.146±0.013 | [a]0.091 |
| MHCC-97L | 6 | 0.134±0.013 | [a]1.000 |
| BEL-7402 | 6 | 0.162±0.006 | [b]0.000 |
| SMMC-7721 | 6 | 0.146±0.013 | [a]0.092 |
| HePG-2 | 6 | 0.151±0.003 | [b]0.009 |
| LO2 | 6 | 0.133±0.006 | -- |

*Each cell line is compared to the LO2 cell line. The mean difference is significant at a level of p=0.05, Data are presented as the mean±SD. [a]$p$>0.05 *vs* $LO_2$, [b]$p$<0.05 *vs* $LO_2$

**Table 4. The Distance between the Cell Fronts on Either Side of the Scratch 24H Later Measured by IPP of the five Different Liver Cancer Cell Lines and the $LO_2$ Cell Line**

| Group | n | 24 H | *p* |
|---|---|---|---|
| MHCC-97H | 6 | 0.1030±0.0060 | [b]0.002 |
| MHCC-97L | 6 | 0.0703±0.0100 | [a]0.052 |
| BEL-7402 | 6 | 0.1500±0.0100 | [b]0.000 |
| SMMC-7721 | 6 | 0.1200±0.0100 | [b]0.000 |
| HePG-2 | 6 | 0.0858±0.0492 | [b]0.002 |
| $LO_2$ | 6 | 0.0152±0.0168 | --- |

*Each cell line is compared with the LO2 cell line. Data are presented as the mean±SD. The mean difference is significant at $p$=0.05. [a]$p$>0.05 *vs* LO2,[b]$p$<0.05, *vs* $LO_2$

invasion compared with the other cancer cell lines ($p$=1.000). The Bel-7402 and HepG-2 cell lines exhibited increased invasion relative to $LO_2$ cell line ($p$<0.05). The SMMC-7721 and MHCC-97H cell lines exhibited moderate invasion ($p$=0.091 and 0.092, respectively), as shown in Figure 2 (C) and in Figure 3 (C) and Table 3.

The microscopy images in Figure 2 (D) demonstrate that the migration abilities differed among the five cancer cell lines ($p$=0.0000). Compared with the $LO_2$ cell line, the elevated Talin-1-expressing cell line, MHCC-97L, exhibited slightly reduced migration ($p$=0.052). The Bel-7402, SMMC-7721, HepG-2 and MHCC-97H cell lines exhibited increased migration compared with $LO_2$ cell line ($p$<0.05), as shown in Figure 2 (D) and in Figure 3 (D) and Table 4.

*The relationship between Talin-1 expression and the colony-forming ability in liver cancer cell lines*

The microscopy images shown in Figure 2 (E) demonstrate that the colony-forming ability differed among the cancer cell lines compared with the $LO_2$ cell line ($p$=0.00017). The high Talin-1-expressing

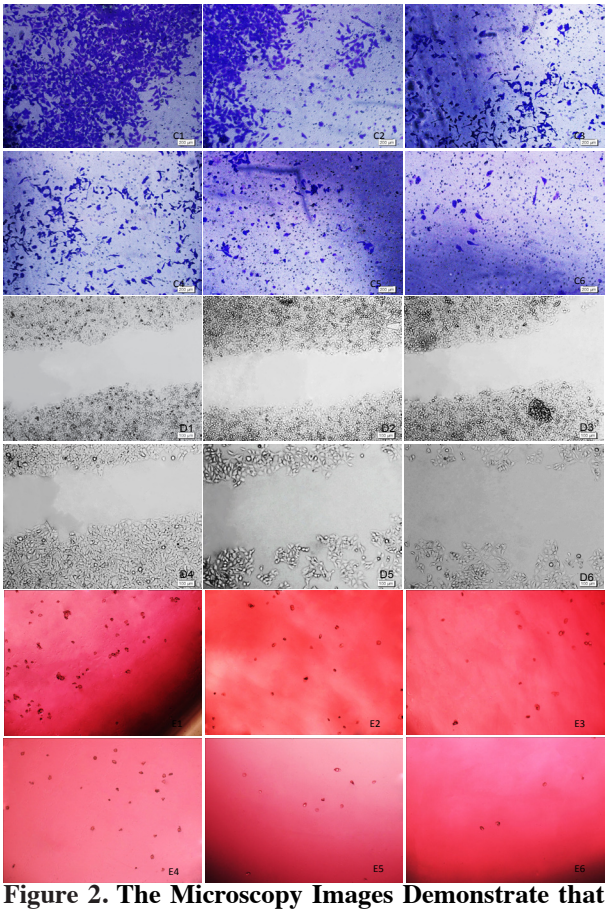


**Figure 2. The Microscopy Images Demonstrate that the Invasion, Migration, and Colony-forming Abilities of the Five Cancer Cell Lines**

**Table 5. The Colony Count and Rate of Clone Formation**

| Group | n | Average | Colony count | Rate (%) | *P* |
|---|---|---|---|---|---|
| MHCC-97H | 24 | 53.46±13.20 | 60 | 150 | [b]0.000 |
| MHCC-97L | 24 | 40.33±7.56 | 52 | 130 | [a]0.185 |
| BEL-7402 | 24 | 61.92±10.59 | 64 | 160 | [b]0.000 |
| SMMC-7721 | 24 | 50.83±10.14 | 57 | 140 | [b]0.000 |
| HePG-2 | 24 | 49.46±6.60 | 54 | 135 | [b]0.000 |
| $LO_2$ | 24 | 34.91±7.11 | -- | -- | -- |

*Each cell line is compared with the $LO_2$ cell line. Data are presented as the mean±SD. The mean difference is significant at $p$=0.05. [a]$p$>0.05,*vs* $LO_2$, [b]$p$<0.05 *vs* $LO_2$

MHCC-97L cell line produced 52 colonies for a colony clone formation rate of 130% ($p$=0.185 and $p$>0.05, respectively). The low Talin-1-expressing BEL- 7402 cell line produced 64 colonies for a colony formation rate of 160%, as shown in Figure 2 (E) and in Figure 3 (E) and Table 5.

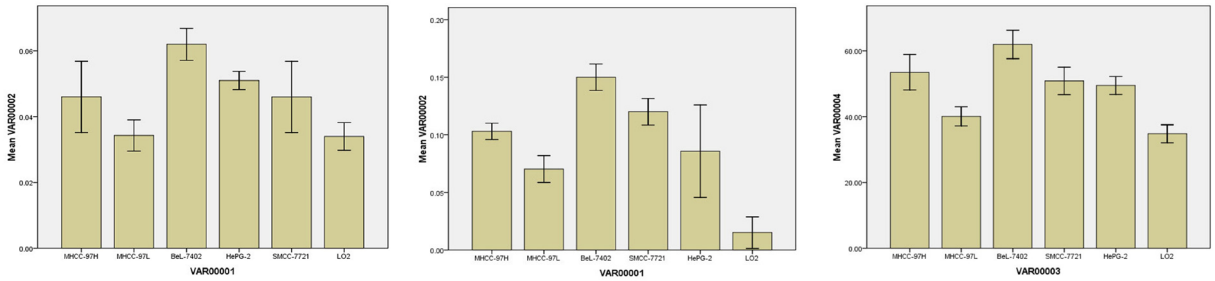


**Figure 3. The Microscopy Images were Analyzed by SPSS 17.0 to Evaluate the Relationship between Talin-1 Expression and the Colony-forming, Invasion, and Migration Abilities of the Liver Cancer Cell Lines**

Different liver cancer cell lines exhibit differential invasion and migration capabilities. Talin-1 is associated with the invasion and migration of human liver cell lines. High Talin-1 expression was correlated with a reduced invasion and migration ability and decreased malignancy. In contrast, decreased Talin-1 expression was correlated with increased invasion and migration.

## Discussion

According to new research, there are many histological subtypes of primary liver cancer: cirrhotic hepatocellular, fibrolamellar carcinoma, hepatocellular carcinoma, combined hepatocellular-cholangiocarcinoma, sarcomatoid hepatocellular carcinoma, undifferentiated carcinoma, lymphoepithelioma-like hepatocellular carcinoma, Intrahepatic cholangiocarcinoma (ICC) and others (Shafizadeh, 2013; Sudarat et al., 2013). The subtypes differ with respect to malignancy and prognosis. Recent studies have argued that cirrhotic HCC is more aggressive than classical HCC. More recent studies have demonstrated that the prognosis in FLM is similar to conventional HCC in a non-cirrhotic liver (Kakar et al., 2005; Shafizadeh, 2013). The 5-year survival rate in FLM is 50-60% in most series, with surgical resectability being the most important prognostic factor (Craig et al., 1980; Lack et al., 1983; Hodgson, 1987; Kakar et al., 2005; Shafizadeh, 2013). Undifferentiated carcinomas are thought to exhibit a worse prognosis than conventional HCC (Ishak et al., 2001; Shafizadeh, 2013). Since the first human HCC cell line was established in 1963 (Chenet et al., 1963), there are more than ten human HCC cell lines have been established (Lou et al., 2004), MHCC-97H and MHCC-97L human liver cancer cells are frequently used in nude mouse models of highly metastatic liver cancers. But pulmonary metastases develop in 100% of MHCC-97H and in 40% of MHCC-97L (Li et al., 2001). HepG-2 cells are derived from human liver cancer cells and are HBsAg–negative (Morris et al., 1982). SMMC-7721 cells originated from a population of cells that were derived from a single clone which expressed IGF-II strongly (Wang et al., 2003). BeL-7402 human liver cancer cells are AFP-positive (Li et al., 2002). From our experiments, we can conclude that different types of liver cancer cells exhibit differing invasion and migration capabilities ($p<0.05$). The invasion and migration abilities of liver cancer cell lines in vitro can predict malignant progression and prognosis.

Talin-1 is a cytoskeleton protein that participates in migration and plays a role in tumor formation, migration, and metastasis in different cancer types. Talin-1 represents a potential diagnostic and prognostic marker that merits further investigation.

Japanese scholars have reported that Talin-1 is upregulated in HCC. However, Talin-1 expression levels in HCC nodules were significantly associated with undifferentiated HCC. A follow-up survey of the examined clinical cases revealed a correlation between Talin-1 upregulation and a shorter time to recurrence after resection, which may be related to the higher rate of portal vein invasion in HCCs with Talin-1 (Kanamori et al., 2011). Jian-Lin Zhang reported that the protein and mRNA expression of Talin-1 in HCC tissues was significantly lower than that in the adjacent non-cancerous tissues and normal liver tissues. In addition, the expression of Talin-1 in HCCs was significantly correlated with pathological differentiation, the integrity of the tumor capsule, portal vein tumor thrombus, and tumor size (Zhang et al., 2011). Egyptian investigators have reported that Serum levels of TLN1 in hepatocellular carcinoma patients were significantly higher compared to the liver cirrhosis (LC) and healthy controls. (Youns MM., et al 2013). The overexpression of Talin-1 has been reported to enhance prostate cancer cell adhesion, migration, and invasion by activating survival signals and conferring resistance to anoikis (Sakamoto et al., 2010). TLN1 overexpression could serve as a diagnostic marker for aggressive phenotypes and a potential target for treating OSCC (Lai et al., 2011). The expression of KIF14 and TLN 1 is a prognostic marker for a better outcome after cytotoxic chemotherapy, and the inhibition of these genes can sensitize KIF14- and TLN1-overexpressing TNBC cells to therapeutic intervention (Singel et al., 2013). In a retrospective study on banked tissue, alpha-actinin and Talin were found to be completely absent in both endometriosis and endometrioid carcinoma tissue (Slater et al., 2007; Zhang et al., 2011).

Our experiment demonstrated that Talin-1 is associated with the invasion and migration of human liver cell lines; elevated Talin-1 expression was correlated with increased invasion and migration and decreased tumor grade. Therefore, Talin-1 may represent a marker of primary liver cancer invasion and migration and the prognosis. The mRNA expression of Talin-1 differed between liver cancer cell lines and normal liver cell lines ($p<0.0001$). The mRNA expression of Talin-1 was higher in the MHCC-97L cell line than in the normal liver cell line ($LO_2$) ($p<0.0001$). The Bel-7402 and HePG-2 cell line exhibited lower expression than the normal liver cell line ($LO_2$) ($p<0.0001$). The MHCC-97H and SMMC-7721 cell lines also exhibited reduced expression compared with the normal liver cell line ($LO_2$) ($p>0.05$).

Our results differ somewhat from the results of Jian-Lin Zhang and Japanese scholars and Egyptian investigators. Compared to liver tissue, normal prostate, breast, uterine, and oral mucosal tissue exhibit low levels of Talin-1 expression. Thus, our research focused on understanding the quantitative relationship between the expression of Talin-1 and tumor invasion and migration. We conclude the following: 1) The majority of primary liver cancer specimens are adenocarcinomas, which are not comparable to the histological subtypes analyzed thus far with respect to Talin-1 expression and liver cancer invasion and migration; 2) Talin-2 may be correlated with invasion and migration in human hepatocellular carcinoma, Although Tln2 is reported to be the ancestral gene (Senetar 2005; Debrand et al., 2012), the specific function of the Talin-2 protein has not been established in any organism. Talin-2 is more widely expressed than originally inferred from northern blots (Monkley et al., 2001; Debrand et al., 2012) and is the most abundant isoform in brain and muscle (praekelt et al., 2012; Debrand et al., 2012).

In vitro, Talin-2 is upregulated during myoblast fusion (Senetar et al., 2007; Debrand et al., 2012), suggesting an important role in muscle development. Talin-2 co-localizes with the muscle-specific β1D-integrin splice variant in myotendinous junctions (Conti et al., 2008; Conti et al., 2009; Debrand et al., 2012). Studies have demonstrated that the N-terminal Talin-2 FERM domain binds to the b1D-integrin with a higher affinity than Talin-1 (Anthis et al., 2010; Debrand et al., 2012). Knockout or knockdown of Talin-1 in cultured cells leads to the upregulation of Talin-2, which compensates for the loss of Talin-1 (Zhang et al., 2008; Kopp et al., 2010; Debrand et al., 2012). Studies on cells in culture clearly establish that Talin-2 can compensate for the loss of Talin-1, and Talin-2 can support cell spreading and FA assembly in Talin-1 knockout or knockdown cells (Zhang et al., 2008; Kopp et al., 2010; Debrand et al., 2012). Our experiment demonstrated the mRNA expression of Talin-2 in five liver cancer cell lines exhibited lower expression than the normal liver cell line ($LO_2$) ($p<0.0001$).

Further study of the role of Talin-1 in liver cancer invasion and migration as well as its influence on liver cancer cell apoptosis is warranted, and the role of Talin-2 in the initiation and progression of primary liver cancer. and should examine its expression in MHCC-97L cells and in TLN1 knockout cells. The role of Talin in liver cancer invasion and migration remains to be further clarified.

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