RESEARCH ARTICLE

Serum Talin-1 is a Potential Novel Biomarker for Diagnosis of Hepatocellular Carcinoma in Egyptian Patients

Mahmoud M Youns[1]*, Abdel Hady A Abdel Wahab[2], Zeinab A Hassan[1], Mohamed S Attia[1]

Abstract

Background: Hepatocellular carcinoma (HCC) is a major cause of cancer mortality worldwide. The outcome of HCC depends mainly on its early diagnosis. To date, the performance of traditional biomarkers is unsatisfactory. Talins were firstly identified as cytoplasmic protein partners of integrins but Talin-1 appears to play a crucial role in cancer formation and progression. Our study was conducted to assess the diagnostic value of serum Talin-1 (TLN1) compared to the most feasible traditional biomarker alpha-fetoprotein (AFP) for the diagnosis of HCC. Methods: TLN1 was detected using enzyme linked immunosorbent assay (ELISA) in serum samples from 120 Egyptian subjects including 40 with HCC, 40 with liver cirrhosis (LC) and 40 healthy controls (HC). Results: ROC curve analysis was used to create a predictive model for TLN1 relative to AFP in HCC diagnosis. Serum levels of TLN1 in hepatocellular carcinoma patients were significantly higher compared to the other groups ($p<0.0001$). The diagnostic accuracy of TLN1 was higher than that of AFP regarding sensitivity, specificity, positive predictive value and negative predictive value in diagnosis of HCC. Conclusions: The present study showed for the first time that Talin-1 (TLN1) is a potential diagnostic marker for HCC, with a higher sensitivity and specificity compared to the traditional biomarker AFP.

Keywords: Hepatocellular carcinoma - Talin-1 - alpha-fetoprotein - liver cirrhosis - marker

Asian Pacific J Cancer Prev, **14 (6)**, 3819-3823

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common tumor and the third most common cause of cancer-related deaths worldwide (El-Serag, 2004; Ferlay et al., 2004). In Egypt, the growing incidence of HCC, which is nearly doubled over the last decade (Hassan et al., 2001; El-Zayadi et al., 2005; Freedman et al., 2006), is attributed to the highest prevalence of hepatitis C virus (HCV) all over the world, ranging from 6 to 28% (Abdel-Aziz et al., 2000; Khattab et al., 2010). HCV increases HCC risk through promoting fibrosis, and finally leading to cirrhosis. HCC develops at an annual rate of 1-4%, when HCV-related cirrhosis is established (El-Serag and Rudolph, 2007).

The incidence and mortality rates for HCC are nearly identical, indicating the overall poor survival of patients with this tumor. Therefore, the most effective treatment relies on the early diagnosis of hepatocellular carcinoma (Bruix and Sherman, 2005).

Alterations in cell-cell adhesion molecules, protein kinases and phosphatases, integrin-associated signaling molecules, or apoptosis regulators can confer resistance to anoikis which is a form of programmed cell death and promote progression to metastasis (Liotta and Kohn, 2004).

Integrins constitute a family of transmembrane receptor proteins composed of heterodimeric complexes of noncovalently linked alpha and beta chains. They function in cell-to-cell and cell-to-extracellular matrix (ECM) adhesive interactions and transduce signals from the ECM to the cell interior and vice versa mediating the ECM influence on cell growth and differentiation. Since these properties implicate integrin involvement in cell migration, invasion, intra- and extra-vasation, and platelet interaction; a role for integrins in tumor growth and metastasis is obvious. These findings are underpinned by observations that integrins are linked to the actin cytoskeleton involving talin, vinculin, and alpha-actinin as intermediaries (Mizejewski, 1999).

Alpha-fetoprotein (AFP) is a serological marker currently available for the detection of hepatocellular carcinoma. Its poor sensitivity renders it unsatisfactory for this purpose and suggests an urgent need for novel biomarkers for early stage HCC detection (Teofanescu et al., 2010).

Talin was the firstly identified cytoplasmic protein partner of integrins (Horwitz et al., 1986). Vertebrates have two talin genes, TLN1 and TLN2, which encode Talin-1 and Talin-2, respectively. Talin-1 is essential for cell adhesion and motility and is the primary talin component of focal adhesions.

*[1]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Ain Helwan, [2]Department of Cancer Biology, National Cancer Institute, Cairo University, Cairo, Egypt *For correspondence: dr.mahmoudyouns@yahoo.com*

It has been shown that, TLN1 binds to the NPXY motif of the β integrin cytoplasmic domains, which can lead to activation of integrin and also affects cell adhesion, spreading and motility (Tadokoro et al., 2003; Zhang et al., 2008). Furthermore, Talin expression is highly associated with endometrioid carcinoma (Slater et al., 2007) and prostate cancer (Sakamoto et al., 2010).

Talin-1 is expressed mainly in the kidney, liver, spleen, stomach, lung and vascular smooth muscle and its overexpression can promote prostate cancer cell adhesion, migration and invasion (Tsujioka et al., 2004; Senetar and McCann, 2005; Senetar et al., 2007). Recently Kanamori et al. (2011) identified Talin-1 by differential Tissue Proteome as a novel molecular marker for HCC progression. However, to our knowledge, its clinical validation as a serum marker for early diagnosis and prognosis of HCC patients has not been investigated.

Materials and Methods

Subjects and samples

One hundred twenty subjects were included in this study and classified into three groups: The first group included patients diagnosed with HCC (mean age±SEM, 51.13±1.216 years; male: female ratio, 1.3:1) at Liver and Endemic diseases department of Al-Kasr Al-Ainy educational hospital, Cairo, Egypt. Diagnosis of HCC was based on elevated AFP levels, the presence of hepatic focal lesion(s) detected by liver ultrasound and confirmed by computed tomography and/or magnetic resonance.

The second group included 40 patients with cirrhosis (mean age±SEM, 49.98±1.37 years; male: female ratio, 1.2:1). Diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic and/or histological criteria.

The last group included 40 healthy individuals served as control (mean age±SEM 48.55±1.53 years; male: female ratio, 1.2:1) were analyzed. Patients with rheumatoid arthritis, alcohol abuse, autoimmune liver diseases and active infection or other malignancies were not included.

Serum samples were collected, aliquoted, stored at -80°C and thawed at the time of assay. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and all patients gave informed consent to participate in the present study.

Detection of HCV

All Serum samples had been screened for HCV using HCV one step test device which is a qualitative membrane based immunoassay for detection of antibodies to HCV in serum or plasma. The membrane is coated with recombinant HCV antigens on the test line region of the device which reacts specifically with HCV antibodies. Presence of a colored line indicates a positive result while its absence indicates a negative one (Abon Biopharm (Hangzhou) Co.Ltd China).

Detection of hepatitis B virus (HBV)

All serum samples had also been screened for HBV using one step Hepatitis B surface antigen (HBsAg) test device which is a qualitative membrane based immunoassay for the detection of HBsAgs in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the device which reacts specifically with HBsAgs and generating a colored line. The presence of this colored line in the test region indicates a positive result while its absence indicates a negative one (Abon Biopharm (Hangzhou) Co.Ltd China).

Blood glucose level (BGL) screening

Random blood glucose levels (RBGL) were screened for all study subjects using commercially available digital glucometer (Bionime blood glucose monitoring system Rightest™).

Baseline characteristics

Serum alanine aminotransferase (ALT) (colorimetric), aspartate aminotransferase (AST) (colorimetric), Alkaline phosphatase (ALP) (colorimetric), gamma glutamyl transferase (GGT) (kinetic), total proteins (colorimetric), albumin (colorimetric) and total bilirubin (colorimetric) levels were determined using commercially available kits for all serum samples. The aspartate aminotransferase/ alanine aminotransferase (AST/ALT) ratio was calculated as (AST/ALT).

Determination of serum AFP

According to the manufacturer's directions, serum AFP levels were measured using the CALBIOTECH human AFP ELISA kits, which were purchased from Modern Egypt Co. Briefly, 25 μl of AFP standards, control and patient's sera were added to the plates pre-coated with AFP antibodies first, then 100 μl of AFP antibody conjugated to horseradish peroxidase were added to all wells, covered and incubated for 60 min at room temperature (18-26ºC). After 3 washes using automatic microplate washer (Stat Fax® 2600), 100 μl of tetramethylbenzidine substrate solution were added to all wells, and incubated for 15 min at room temperature in dark, followed by the addition of 50 μl of stop solution. Absorbances were measured within 15 min after adding stop solution at 450 nm using Microplate reader (Sunrise™ Tecan Group Ltd.).

Determination of serum Talin-1

According to the manufacturer's directions, serum Talin-1 levels were measured using human Talin-1 ELISA kits (WKEA Med Supplies Corp, New York, Usa) which were purchased from Modern Egypt Co. Briefly, 50 μl of serially diluted standards, 5-fold diluted patient's sera (10μl serum: 40 μl sample diluents) were added to the plates pre-coated with Talin-1 antibodies first and incubated for 30 min at 37ºC. After five washes with the diluted buffer concentrate reagent using automatic microplate washer (Stat Fax® 2600), 50 μl of Talin-1 antibody conjugated to horseradish peroxidase were added to all wells except blank wells. After incubation for 30 min at 37ºC and five washes, 50 μl of substrate A followed by 50 μl of substrate B were added to each well and incubated for 15 min at 37ºC followed by the addition of stop solution to each well. Absorbances were measured within 15 min after adding stop solution at 450 nm using microplate reader (Sunrise™ Tecan Group Ltd.).

Statistical analysis

Statistical analyses were performed using Graph pad prism 5 software. Median, range, mean, standard deviation and standard error were used for descriptive statistics, as appropriate. Categorical variables were tested with Fisher's exact test or χ^2 test. Continuous variables were tested with Student t-test or analysis of variance (ANOVA test). Comparison of plasma or serum TLN1 levels and clinical characteristics among the three groups of subjects were analyzed using Kruskal-Wallis test (non-parametric ANOVA test), and Mann-Whitney U test (non-parametric student t test). All tests were two-tailed and statistical significance was assessed at the 0.05 level. Correlation between serum levels of TLN1 and other biochemical variables were analyzed using Spearman's correlation coefficient. Receiver operating characteristics (ROC) analysis was used to evaluate the diagnostic value of TLN1 and AFP to identify the optimal cut off values. Sensitivity and specificity, positive and negative predictive values of TLN1 and AFP were profiled by curves. The Statistical Package for the Social Sciences version 16 (SPSS, Inc., Chicago, IL, USA) was used for ROC curve.

Results

Patient characteristics

Table 1 shows the socio-demographic and clinical characteristics of all study groups expressed as the mean±SEM. Age, gender, body mass index (BMI), residence, showed no significant difference between groups. No significant difference were found considering smoking habit, incidence of diabetes mellitus (DM), hepatitis C Virus (HCV), hepatitis B virus (HBV) or both and ascitis between liver cirhosis (LC) and hepatocellular (HCC) groups. A significant difference has been shown among all studied groups with regard to measured liver function tests and tumor markers.

Serum Talin-1 level in patients with HCC

Our results showed that, the median serum Talin-1 levels were significantly higher in the HCC group compared to LC and HC groups as determined by non-parametric Kruskal-Wallis test followed by Dunn's Multiple Comparison Test (p-value<0.0001) (Figure.1). Additionally, serum TLN1 levels were not significantly affected by sex differences in males vs. females of the same group, in HC (1.11±0.45vs1.08±0.49), LC (24.14±8.30vs. 8.80±2.23) and HCC (60.87±3.25vs. 62.65±3.93) at (p<0.05) using non-parametric Mann Whitney student t test.

Correlation between serum Talin-1 concentration and baseline characteristics within LC and HCC groups

No significant correlations have been found between serum levels of TLN1 with the other biochemical variables within LC and HCC groups (Table 2). Except for a weak negative correlation between serum TLN1 and BMI, and a weak positive correlation between serum TLN1 and ALT enzyme activity within HCC group.

ROC curve analysis revealed a very high area under the curve (AUC) [equals 1] at a confidence interval 95% for serum TLN1, discriminating HCC patients from HC subjects and demonstrated absolute sensitivity and specificity of serum TLN1 in differentiating HCC patients from normal HC subjects (Figure 2).

Furthermore as a diagnostic tool between HCC patients and LC patients, ROC curve analysis of serum TLN1

Table 1. Demographic, Clinical and Biochemical Characteristics of the Study Subjects Groups

Variables[x]	HCC (n=40)	LC (n=40)	HC (n=40)	P[a] value	P[b] value
Age(years)	51.13±1.216	49.98±1.37	48.55±1.53	0.3	0.5
Gender (%)				1.0	1.0
Males	23(57.5%)	22 (55%)	22 (55%)		
Females	17(42.5%)	18 (45%)	18 (45%)		
BMI (kg/m^2)	31.30±0.73	30.35±0.79	28.40±0.70	0.7	0.4
Residence (%)				0.9	0.8
Urban	32 (80%)	30 (75%)	31 (77.5%)		
Rural	8 (20%)	10 (25%)	9 (22.5%)		
Smoking habit (%)				0.0046	0.1
Smokers	18 (45%)	10 (25%)	5 (12.5%)		
Non-smokers	22 (55 %)	30 (75%)	35 (87.5%)		
DM (%)				0.0082	0.6
Diabetic	6 (15%)	9 (22.5%)	0 (0%)		
Non-diabetic	34 (75%)	31 (77.5%)	40(100%)		
HCV (%)				0.0001	0.6
Positive	32 (80%)	29 (72.5%)	0 (0%)		
Negative	8 (20%)	11 (27.5%)	40 (100%)		
HBV (%)				0.0067	1.0
Positive	8 (20%)	9 (22.5%)	0 (0%)		
Negative	32 (80%)	31 (77.5%)	40 (100%)		
HCV and HBV	4 (10%)	6 (15%)	0 (0%)	0.05	0.7
Ascitis (%)				0.0001	0.6
Present	21 (52.5%)	18 (45%)	0 (0%)		
Absent	19 (47.5%)	22 (55%)	40 (100%)		
RBGL(mg/dl)	121.5±12.64	123.8±10.68	124.4±3.46	0.0007	0.6
ALT(U/ml)	70.13±2.65	47.63±1.39	21.55±1.12	0.0001	0.0001
AST(U/ml)	137.4±5.30	75.30±2.03	16.60±0.70	0.0001	0.0001
AST/ALT ratio	2.023±0.08	1.59±0.03	0.80±0.02	0.0001	0.0001
ALP(IU/L)	125.00±1.58	98.95±4.10	64.15±3.27	0.0001	0.0001
GGT(U/L)	102.80±1.47	56.60±4.06	25.95±1.82	0.0001	0.0001
Total Bilirubin (mg/dl)	2.36±0.10	1.13±0.05	0.65±0.03	0.0001	0.0001
Total protein (g/dl)	5.44±0.08	6.22±0.17	7.79±0.16	0.0001	0.0002
Albumin (g/dl)	2.29±0.06	2.77±0.11	3.97±0.05	0.0001	0.0005
AFP (ng/ml)	123.3±35.59	80.2±26.60	14±12.47	0.0001	0.0064
Talin-1 (ng /ml)	61.63±2.47	17.24±4.78	1.1±0.32	0.0001	0.0001

*P[a]: P value among all study groups, P[b]: P value between LC and HCC groups P<0.05 is considered significant. **Hepatocellular Carcinoma: HCC, Liver Cirrhosis: LC, Healthy Control: HC

Table 2. No Significant Correlations have been Found between Serum Levels of TLN1 with the Other Biochemical Variables Within LC and HCC Groups

Parameter	LC Group		HCC Group	
	Spearman correlation		Spearman correlation	
	Coefficient (r)	P-value	Coefficient (r)	P-value
Age	0.07	0.65	-0.15	0.34
BMI	-0.18	0.25	-0.31*	0.04
RBGL	-0.01	0.95	-0.09	0.57
ALT	0.27	0.09	0.33*	0.03
AST	0.27	0.09	0.10	0.50
AST/ALT ratio	-0.04	0.79	-0.20	0.21
ALP	0.10	0.53	0.27	0.08
GGT	-0.04	0.76	0.04	0.77
T.B	-0.11	0.49	0.17	0.28
T.P	0.30	0.05	0.05	0.72
Albumin	0.13	0.43	0.19	0.23
AFP	-0.04	0.81	0.02	0.86

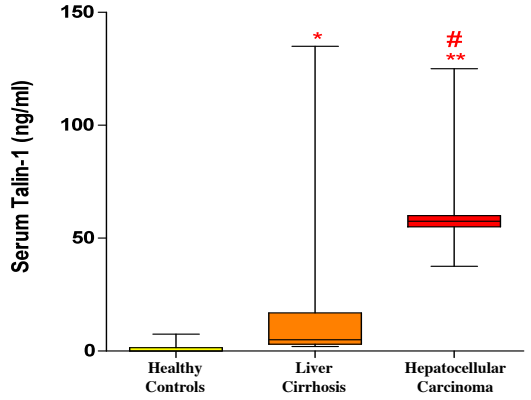
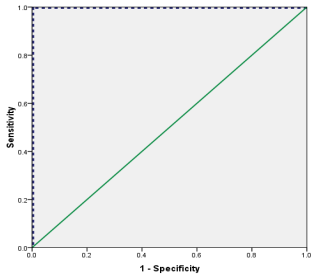
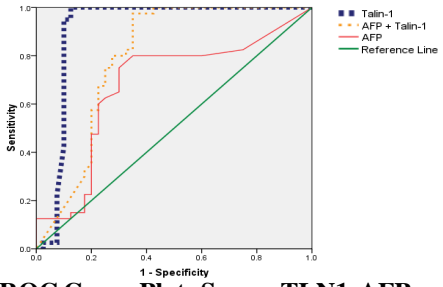


Figure 1. Serum TLN1 Levels in Patients with Hepatocellular Carcinoma (HCC), Liver Cirrhosis (LC), and Healthy Controls (HC). Bars are median values of each disease group. The median serum TLN1 level in HCC was significantly higher than in LC or healthy controls (p-value<0.0001 by non-parametric Kruskal-Walis test followed by Dunn's multiple comparison test). *significant difference when compared with HC group (p<0.0001). **significant difference when compared with HC group (p<0.0001). #significant difference when compared with LC group (p<0.0001)



Figureure 2. ROC Curve Plots Serum TLN1 in Discriminating HCC Patients from HC Subjects



Figureure 3. ROC Curve Plots Serum TLN1, AFP and Combination of Them in Discriminating HCC Patients from Those with LC

showed higher AUC (0.90) compared to that of AFP (0.67). Moreover AUC of seum TLN1 alone was higher than that of its combination with AFP (0.79) (Figure 3).

Performance of biomarkers

Table 3 shows sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of serum Talin-1, AFP and combination of both in hepatocellular carcinoma (HCC) patients relative to the liver cirrhosis (LC) group.

Discussion

Liver cancer (LC) is the sixth most common cancer worldwide and the third most common cause of cancer

Table 3. The Optimal Cut Off Values and the Diagnostic Accuracy of TLN1, AFP and their Combination in Diagnosis of HCC From LC

Tumor Marker	Cut off ng/ml	Sensitivity %	Specificity %	PPV %	NPP %
Talin-1	33.75	100	87	88	100
AFP	9	80	65	69	76
Talin-1+AFP	AFP=9, Talin-1=33.75	100	57	70	100

mortality, with more than 500,000 deaths annually (Parkin et al., 2001; 2005). Hepatocellular carcinoma (HCC), is rarely detected early and is usually fatal within a few months of diagnosis (Thomas and Zhu, 2005).

A recently published study indicated that the incidence rates of HCC had been tripled in the United States from 1975 through 2005 (Altekruse et al., 2009). In Egypt HCC incidence rates have been doubled over the last ten years (Hassan et al., 2001; El-Zayadi et al., 2005; Freedman et al., 2006) and that is attributed to the growing HCV incidence (Abdel-Aziz et al., 2000; Khattab et al., 2010).

Currently, early diagnosis of HCC is the most important step in HCC management. Most imaging techniques help to discover HCC after considerable time of onset of tumor. In most instances, oncologists depend on AFP as the commonest and feasible marker for assessing HCC in addition to imaging. However, AFP is not completely reliable marker in early HCC diagnosis, prevention or therapy due to its low specificity and sensitivity. Liver biopsy is always considered as an invasive procedure, so chemical findings are still greatly appreciated (Makuuchi et al., 2008).

The ideal hepatic tumor biomarkers should possess high specificity and sensitivity not to be detected in cirrhosis. It should be easily accessible, easily measurable, minimally invasive, inexpensive, accurate, acceptable to patients and physicians (Mendy and Walton, 2009).

Talin is a large (≈270-kDa) cytoskeletal adaptor protein that is important component of focal adhesion complexes of adherent cells (Critchley, 1999). It has been shown that Talin-1 overexpression enhanced prostate cancer cell adhesion, migration, and invasion by activating survival signals and conferring resistance to anoikis. ShRNA (short hairpin RNA)-mediated Talin-1 loss led to a significant suppression of prostate cancer cell migration and trans endothelial invasion in vitro and a significant inhibition of prostate cancer metastasis in vivo (Sakamoto et al., 2010).

However, TLN1 expression in HCC is still controversial. Recently, TLN1 has been identified by differential Tissue Proteome as a novel molecular marker for HCC progression and has revealed that its up regulation is associated with HCC progression showing that it may serve as a prognostic marker (Kanamori et al., 2011).

On the other hand, Zhang et al. (2011) have proved that TLN1 is down-regulated in HCC liver tissues when compared with adjacent non-cancerous liver tissues or with control liver tissues by immuno- histochemistry and real time PCR.

In our study we found that serum TLN1 levels were significantly higher in the HCC group than in the LC group or in the normal control group. Besides, ROC curve analysis showed AUC for TLN1 higher than that of

AFP alone or in combination with TLN1. Hence, TLN1 showed higher sensitivity, specificity, PPV, NPV in HCC differentiation from patients with liver cirrhosis.

Our study agrees with those of Bessa et al. (2010) and Attallah et al. (2011) concerning AFP which showed AUC (AUC 0.71 and 0.7) in predicting HCC in Egyptian population. However our study showed the superiority of serum TLN1 over AFP in diagnosis of HCC.

In conclusion, The present study is the first demonstrating a potential utility of serum TLN1 in early HCC detection by comparing its serum levels with AFP in HCC, LC patients, and control healthy subjects.

Furthermore, it suggests that serum TLN1 levels might serve as a potential candidate biomarker for HCC, with superior diagnostic accuracy than AFP or other routine liver markers at least under the current settings. The ultimate diagnostic utility and implication of serum TLN1 in HCC needs to be validated in other large multicenter cohort studies.

Aknowledgement

We would like to thank the staff members at the National cancer institute (NCI) for their expert technical help during subject recruitment, samples collections and practical analyses. We are also grateful to our colleagues at faculty of pharmacy, Helwan University especially, Ahmed M. Abd-Elmohsen and Shahenda Mahgoub for their scientific help and support.

References

Abdel-Aziz F, Habib M, Mohamed MK, et al (2000). Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. *Hepatology*, **32**, 111-5.

Altekruse S, McGlynn K Reichman M (2009). Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol*, **27**,1485-91.

Attallah AM, El-Far M, Abdel Malak C, et al (2011). Evaluation of cytokeratin-1 in the diagnosis of hepatocellular carcinoma. *Clinica Chimica Acta*, **412**, 2310-5.

Bessa S, Elwan N, Suliman G, El-Shourbagy S (2010). Clinical significance of plasma osteopontin level in Egyptian patients with hepatitis C virus-related hepatocellular carcinoma. *Arch Med Res*, **41**, 541-7.

Bruix J and Sherman M (2005). Management of hepatocellular carcinoma. *Hepatology*, **42**, 1208-36.

Critchley DR, Talin In Kreis T, Vale R (1999). Guidebook to the extracellular matrix, anchor, and adhesion proteins. Oxford University Press, pp. 82-85, Oxford.

El-Serag HB (2004). Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology*, **127**, 27-34.

El-Serag HB, Rudolph KL (2007). Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*, **132**, 2557-76.

El-Zayadi AR, Badran HM, Barakat EM, et al (2005). Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J Gastroenterol*, **11**, 5193-8.

Ferlay J, Bray F, Pisani P (2004). GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC Press; Lyon: 2004. IARC CancerBase No. 5. version 2.0.

Freedman L, Edwards B, Ries L (2006). Cancer incidence in four member countries (Cyprus, Egypt, Israel, and Jordan) of the middle east cancer consortium (MECC) compared with US SEER. National Cancer Institute. Bethesda, MD: NIH Pub. No. 06-5873.

Hassan MM, Zaghloul AS, El-Serag HB (2001). The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. *J Clin Gastroenterol*, **33**, 123-6.

Horwitz A, Duggan K, Buck C, et al (1986). Interaction of plasma-membrane fibronectin receptor with talin - a transmembrane linkage. *Nature*, **320**, 531-3.

Kanamori H, Kawakami T, Effendi K, et al (2011). Identification by differential tissue proteome analysis of Talin-1 as a novel molecular marker of progression of hepatocellular carcinoma. *Oncology*, **80**, 406-15.

Khattab MA, Eslam M, Sharwae MA (2010). Seroprevalence of hepatitis C and B among blood donors in Egypt: Minya Governorate, 2000-2008. *Am J Infect Control*, **38**, 640-1.

Liotta LA, Kohn E (2004). Anoikis: cancer and the homeless cell. *Nature*, **430**, 973-4.

Makuuchi M, Kokudo N, Arii S, et al (2008). Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res*, **38**, 37-51.

Mendy M, Walton R (2009). Molecular pathogenesis and early detection of hepatocellular carcinoma-perspectives from West Africa. *Cancer Lett*, **286**, 44-51.

Mizejewski GJ (1999). Role of integrins in cancer: survey of expression patterns. *Proc Soc Exp Biol Med*, **222**, 124-38.

Parkin D, Bray F, Ferlay J, Pisani P (2001). Estimating the world cancer burden: Globocan 2000. *Int J Cancer*, **94**, 153-6.

Parkin D, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Sakamoto S, McCann RO, Dhir R, Kyprianou N (2010). Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance. *Cancer Res*, **70**, 1885-95.

Senetar M, Moncman C, McCann R (2007). Talin2 is induced during striated muscle differentiation and istargeted to stable adhesion complexes in mature muscle. *Cell Motil Cytoskeleton*, **64**, 157-73.

Senetar MA, McCann RO (2005). Gene duplication and functional divergence during evolution of the cytoskeletal linker protein talin. *Gene*, **362**, 141-52.

Slater M, Cooper M, Murphy CR (2007). The cytoskeletal proteins alpha-actinin, Ezrin, and talin are De-expressed in endometriosis and endometrioid carcinoma compared with normal uterine epithelium. *Appl Immunohistochem Mol Morphol*, **15**, 170-4.

Tadokoro S, Shattil SJ, Eto K, et al (2003). Talin binding to integrin beta tails:a final common step in integrin activation. *Science*, **302**, 103-6.

Teofănescu I, Gologan E, Stefanescu E, Balan G (2010). Surveillance of cirrhosis for hepatocellular carcinoma--clinical validation of new serological biomarkers for improved diagnosis. *Rev Med Chir Soc Med Nat Iasi*, **114**, 39-46.

Thomas M, Zhu A (2005). Hepatocellular carcinoma: the need for progress. *J Clin Oncol*, **23**, 2892-9.

Tsujioka M, Yoshida K, Inouye K (2004). Talin B is required for force transmission in morphogenesis of dictyostelium. *EMBO J*, **23**, 2216-25.

Zhang JL, Qian YB, Zhu LX, Xiong QR (2011). Talin1, a valuable marker for diagnosis and prognostic assessment of human hepatocelluar carcinomas. *Asian Pac J Cancer Prev*, **12**, 3265-9.

Zhang X, Jiang G, Cai Y, et al (2008). Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat Cell Biol*, **10**, 1062-8.