**Heat Shock Proteins as Diagnostic Markers for Hepatocellular Carcinoma: A Novel Approach**

Amber Ehsan Faquih (A. E. Faquih)1, Ahsan Zil E Ali (A. Zil-E-Ali)2, Anam Waseem (A. Waseem)3, Zubair Ahmed (Z. Ahmed)4, Rooha Tariq (R. Tariq)5

1 Research Assistant, Emory University, Atlanta, US. 2,3,4 FMH College of Medicine, Lahore, Pakistan. 5Federal Medical and Dental College, Islamabad, Pakistan

**Abstract**

Hepatocellular Carcinoma (HCC) is a malignant tumor of the liver with massive morbidity and mortality. Early diagnosis and treatment is necessary to eliminate the disease and to prevent its remission. This study focuses on the role of Heat Shock Proteins as a potential tumor marker for HCC. It is shown to be helpful in differentiating the metastatic and non-metastatic lesions as well as in determining the extent and properties of the tumor. Here, we look at the possibility of using HSPs to monitor the response of the tumor to various medical and surgical interventions and as an early marker specific to HCC.

**Keywords:** Hepatocellular Carcinoma (HCC), Heat Shock Proteins (HSP), tumor marker,

**Introduction**

Hepatocellular Carcinoma (HCC) accounts for 80% of the total carcinomas of the liver. It is the sixth most common cancer in the world [1], 5th among the men & 8th among the women. HCC is the third most common cancer in the region of Asia-Pacific and is the primary liver cancer in many countries. The incidence is higher in men especially above the age of 40. The most common cause of HCC in Asia is Hepatitis B & C [2]. HCC is a very important health problem because of its associated morbidity and mortality. Although many advancements have been made in healthcare system including both diagnosis and treatment, it still accounts for a major burden on our health care system. New effective surveillance and screening programs cannot only decrease morbidity and mortality of patients but can also lead to a better life expectancy of people suffering from Hepatitis B and C.

HSPs are a family of highly specialized proteins that play an important role during cellular stress response [3]. These proteins are being widely explored with their relation to hepatocellular carcinoma in order to achieve a major breakthrough in the diagnosis as well as prognosis of HCC.

**Heat Shock Proteins**

Heat Shock Proteins also referred as stress proteins, are present in all living cells. They have a specialized role in folding and unfolding of proteins, transportation of proteins, control of cell cycle and signaling pathways and protect against apoptosis. They are a large family of proteins which are further classified into smaller subunits according to their molecular weight e.g. HSP 10, HSP 40, HSP 27, HSP 90 etc.

Recently, the role of heat shock proteins is also found in antigen presentation to class I and II of MHC. They can stimulate antigen presenting cells of the immune system i.e. macrophages and dendritic cells. They can also be induced in response to environmental stress to minimize cellular damage and promote cellular survival[4]. The induction of HSP is an important mechanism to help cancer cells adapt to stress conditions. HSP overexpression is documented is many cancers because they protect cancer cells from stress in the tumor environment. Therefore, they are related to poor prognosis and account for treatment resistance as they are protected cancer cells. Upregulation of heat shock proteins can serve as important diagnostic and therapeutic marker. Targeted therapy towards HSP can result in the major breakthrough in the treatment of HCC as well as its prognosis. HSP 27, 70 and 90 are closely related to human malignancies along with clusterin, a small molecule that works in a manner similar to small HSP.

HSP 27 is found in many malignancies. It plays a vital role in inhibition of apoptosis, RNA splicing, DNA repair and degradation of oxidized protein etc. HSP 70 is usually maintained at low levels but is induced under stress conditions including many cancer e.g. breast colon etc. It plays a crucial role in the cellular apoptotic pathway. HSP 90 has a role of paramount importance in anti-apoptosis and pro metastasis. Clustering is also found in many kinds of cancers and is linked to metastasis and epithelial to mesenchymal transition.

**Role of Heat Shock Proteins in Hepatocellular Carcinoma**

HSP have some important role in HCC that can be used in specific targeted therapy involving HSPs. They have a vital role in apoptosis, therapeutic resistance and invasion & metastasis of carcinoma.

* **Apoptosis**

HSP 27 exhibits its cytoprotective effect by inhibiting apoptosis as it inhibits many apoptotic pathways. It also supports cell survival by inhibiting cytochrome c. In HCC decreased level of HSP27 leads to apoptosis of affected cells. Decrease levels of HSP 90 are linked with increased levels of p53 resulting in apoptosis. Clustering protects HCC from endoplasmic stress-induced apoptosis.

* **Therapeutic Resistance**

Therapeutic Resistance is a major obstacle in the treatment of HCC. HSP 27 is upregulated by chemotherapy and results in inhibition of apoptosis. Similar is the case with HSP 90 and clusterin. A major breakthrough in the therapeutic regime of HCC can be achieved by specific targeted therapy against them.

* **Invasion & Metastasis**

HSP 27 and clustering overexpression is associated with tumor metastasis. HSP 70 is also an important tumor migration. Same is the case with HSP 90.HSP27 exhibit their cellular protective response due to their anti-apoptotic properties. Increased levels of HSP 27 are observed in HCC cells as well as in chronic Hepatitis B infected cells. HSP 27 is an ATP independent chaperon so small siRNA can be used in its modulation to produce pro-apoptotic, anti-metastatic and chemo-sensitized effects. OGX-427 is a second generation antisense oligonucleotide that targets HSP27 and effectively suppresses it.

HSP 70 is found to be higher in HCC effected cells than in normal human cells. It can serve as a sensitive marker to differentiate HCC from dysplastic nodules, both low and high grade, hence leading to early HCC detection. In addition to HSP 70 two other marker glypican-3, and glutamine synthesize have shown to be important in the early diagnosis of HCC [5]Increased HSP 70 is also associated with poor prognosis as well as portal vein and vascular invasion.17‐(Demethoxy)‐17‐allylamino geldanamycin (17‐AAG) is also reported to prevent ATP binding to HSP90 through steric inhibition thus resulting in promoting apoptosis. Clusterin is similar to HSP 27, ATP independent so it is also targeted by antisense oligonucleotide specifically OGX-11 that suppresses clusterin levels and promoting apoptosis of affected cells.

**Outcomes of Clinical Trials for Efficacy of Heat Shock Proteins as Tumor Markers**

It showed the rate of expression of phosphorylated HSP27 expression was higher in HCV antibody positive patients as compared to hepatitis B surface antigen positive Patients. Secondly, HSP 27 expression was associated with larger tumor size and portal vein invasion[6]. Expression levels of heat shock protein 20 decreases in parallel with tumor progression in patients with hepatocellular carcinoma. Levels of HSP 20 expressed in healthy cells was significantly higher as compared to HCC affected cells. No correlation was found between phosphorylated HSP27 and HSP20 [7]. Diagnostic Value of HSP70, Glypican 3, and Glutamine Synthetase in Hepatocellular Nodules in Cirrhosis` HSP70 was detected in almost all HCC effected cells. The sensitivity and specificity of HSP 70 were found to be 73.58% and 98.08% respectively. Glypican 3 was found in the majority of tumor cells with its number increasing with tumor dedifferentiation. Glutamine Synthetase overexpression was only seen in High-Grade Dysplastic Nodules(HGDN). Combination of all these factors to differentiate between HGDN and HCC-G1 was also found[5] [8].

HSP70 is associated with tumor size, stage and portal vein invasion. 71.9% of HCC cells were observed to express HSP70 as compared to 12.1% in non-neoplastic liver cells, but still, it cannot predict overall survival of HCC. HSP27 is overall independent prognostic markers that can determine prognosis and recurrence of HCC. Level of HSP27 is significantly raised in HCC cells as compared to normal cells as well as cells with HBV. Thus HSP70 and HSP27 can both be further investigated and can play a potential role in diagnosis as well as prognostic markers of HCC.HSP as the main modality or an integral work up for HCC diagnosis and prognosis. HSP70 can become an important target to increase the sensitivity of cells to anti-tumor drugs, so further workup can be along these lines to develop its role in tumor prognosis and early differentiation.

HSP 27 was mostly associated with hepatitis virus and can be effectively used as a diagnostic marker. Level of HSP20 are reported to decrease with increase in the stage of HCC and may have a suppressive effect on HCC progression.

**Discussion**

Hepatocellular carcinoma(HCC) comprises 80% of primary liver cell carcinoma, incidence wise and only 30-40% of diagnosed patients with HCC are at the curable stage[3]. Therefore the early diagnosis, differentiation, and detection of recurrence are very important in decreasing mortalities from this cancer.

First, differentiating early stage HCC from other liver pathologies is very important and we found that Heat-shock Proteins(HSP) are playing a crucial role in differentiating HCC from others liver disease according to new researchers, like HSP70, a type of heat shock protein, helps in early diagnosis and differentiation of precancerous lesion from Adenomatous hyperplasia , Atypical adenomatous hyperplasia and non-liver cancerous lesions [9]. It also differentiates well-differentiated, small HCC from high grade dysplastic nodules[3] typical vs atypical carcinoma[10] and early HCC from advanced carcinoma (alone) and in combination with other markers like p53 [11][12] and with other biomarkers like GS and Glypican-3 it can help differentiate dysplastic nodules from HCC with a sensitivity of 72% and specificity of 100% [13].

Few other forms of HCC including HSP70 helps in curing HCC and they are being used as therapeutic targets like HSP 70 increases the sensitivity of tumor cells for anti-tumor drugs and helps in treating HCC [14] HSP 20 also being used for therapeutic purpose in HCC especially in tumors with invasion[15]. Now the other one is HSP 27, this HSP can alone tell the overall risk of survival in HCC and metastasis of HCC [3][16]. It is found to be elevated in early HCC with a positive immunoreactivity of 100% for HCC and the levels are particularly related to underlying HCV induced liver cirrhosis[17]. HSF 1 also helps in prognosis and being studied as a therapeutic target in HCC [18]. Other Heat Shock proteins are also being studied to play a role in diagnosis, prognosis, and therapy of HCC [19]. According to tumor markers studies for HCC, there are two HSPs, HSP70 was positively correlated with tumor size, portal vein invasion, and tumor stage, while HSP27 was only associated with HCC which are infected by hepatitis Virus. In HCC, the overexpression of HSP70 and HSP27 promotes tumor growth and metastasis. Furthermore, the expression of HSP70 is correlated with differentiation and apoptosis of tumor cells. It promotes tumor cell growth by stabilizing cyclin D1 and suppresses the apoptosis of tumor cells by inhibiting the p53 pathway. Thus, HSP70 and HSP27 are potential markers for HCC and should be further investigated.

In particular, HSP70 has been identified as a potentially sensitive marker to differentiate early HCC from precancerous lesions. Heat Shock Proteins should be studied as diagnostic marker of HCC as they are good in early diagnosis, in detecting precancerous lesions and also for prognosis, size of tumor and metastasis, the sensitivity and specificity of HSPs in detecting HCC were identified as 57.5 and 85%, respectively as compared to other potential and regular tumor markers like AFP L3 which detects small tumor less than 2cm more effectively than AFP with Sensitivity 96% Specificity 92% and it also helps in prognosis but usefulness is limited to population with high normal AFP level, another tumor marker des gamma carboxy-prothrombin(DCP) is more accurate in diagnosing HCC than AFP and AFP L3 also good in differentiating HCC from other liver pathology but cannot effectively detect small tumors as by AFP-L3 and AFP, so improve Sensitivity when combining with AFP[11][20].

**Conclusion**

In the above discussion, it can be seen that Heat Shock Proteins can prove to be very promising when it comes to determining the extent, characteristics, sensitivity to anti-cancer drugs and metastasis of HCC. It will be very beneficial to Study them in Diagnosing HCC considering their role in early detection, differentiation, and recurrence of HCC.

**Bibliography**

1. Flores A, Marrero JA. Emerging Trends in Hepatocellular Carcinoma: Focus on Diagnosis and Therapeutics Clin Med Insights Oncol

. 2014;8:71-76. doi: 10.4137/CMO.S9926. eCollection 2014.

2. Zhu RX, Seto W-K, Lai C-L, Yuen M-F. Epidemiology of Hepatocellular Carcinoma in the Asia-Pacific Region.Gut Liver 2016;10(3):332-339. doi:10.5009/gnl15257.

3. [Wang C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wang%2520C%255BAuthor%255D&cauthor=true&cauthor_uid=26853533)1, [Zhang Y](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%2520Y%255BAuthor%255D&cauthor=true&cauthor_uid=26853533)1, [Guo K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Guo%2520K%255BAuthor%255D&cauthor=true&cauthor_uid=26853533)2, [Wang N](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wang%2520N%255BAuthor%255D&cauthor=true&cauthor_uid=26853533)1, [Jin H](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jin%2520H%255BAuthor%255D&cauthor=true&cauthor_uid=26853533)1, [Liu Y](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%2520Y%255BAuthor%255D&cauthor=true&cauthor_uid=26853533)2,3 et.al Heat shock proteins in hepatocellular carcinoma: Molecular mechanism and therapeutic potential [Int J Cancer.](https://www.ncbi.nlm.nih.gov/pubmed/26853533/) 2016 Apr 15;138(8):1824-34 doi: 10.1002/ijc.29723

4. Murshid A, Gong J, Calderwood SK. The role of heat shock proteins in antigen cross presentation. Front Immunol. 2012;3:63. doi: 10.3389/fimmu.2012.00063

5. Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E et,al Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. Hepatology. 2007 Mar 1;45(3):725-34. [doi 10.1002/hep.21531](https://doi.org/10.1002/hep.21531)

6. Eto D, Hisaka T, Horiuchi H, Uchida S, Ishikawa H, Kawashima Y, Kinugasa T, Nakashima O, Yano H, Okuda K, Akagi Y. Expression of HSP27 in hepatocellular carcinoma. Anticancer research. 2016 Jul 1;36(7):3775-9.

7. Noda T, Kumada T, Takai S, Matsushima-Nishiwaki R, Yoshimi N, Yasuda E, Kato K, Toyoda H, Kaneoka Y, Yamaguchi A, Kozawa O. Expression levels of heat shock protein 20 decrease in parallel with tumor progression in patients with hepatocellular carcinoma. Oncology reports. 2007 Jun 1;17(6):1309-14.  Doi 10.3892

8. Tremosini S1, Forner A, Boix L, Vilana R, Bianchi L, Reig M et.al .Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. . Gut. 2012 Oct;61(10):1481-7 doi: 10.1136/gutjnl-2012-303541

9. Chuma M, Sakamoto M, Yamazaki K, Ohta T, Ohki M, Asaka M, Hirohashi S. Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. Hepatology. 2003 Jan 1;37(1):198-207 <https://doi.org/10.1053/jhep.2003.50022>

10. Nguyen TB, Roncalli M, Di Tommaso L, Kakar S. Combined use of heat-shock protein 70 and glutamine synthetase is useful in the distinction of typical hepatocellular adenoma from atypical hepatocellular neoplasms and well-differentiated hepatocellular carcinoma. Mod Pathol. 2016 Mar;29(3):283. doi:10.1038/modpathol.2015.162

11. Kim JU, Shariff MI, Crossey MM, Gomez-Romero M, Holmes E, Cox IJ, Fye HK, Njie R, Taylor-Robinson SD. Hepatocellular carcinoma: review of disease and tumor biomarkers. World J Hepatol.2016 Apr 8;8(10):471. doi:  [10.4254/wjh.v8.i10.471](https://dx.doi.org/10.4254%252Fwjh.v8.i10.471)

1. ang, Z., Gou, W., Liu, M., Sang et.al 2015. Expression of P53 and HSP70 in chronic hepatitis, liver cirrhosis, and early and advanced hepatocellular carcinoma tissues and their diagnostic value in hepatocellular carcinoma: An immunohistochemical study Med Sci Monit 2015, 21, p.3209. doi:  [10.12659/MSM.895592](https://dx.doi.org/10.12659%252FMSM.895592)

13. Nguyen TB, Roncalli M, Di Tommaso L, Kakar S. Combined use of heat-shock protein 70 and glutamine synthetase is useful in the distinction of typical hepatocellular adenoma from atypical hepatocellular neoplasms and well-differentiated hepatocellular carcinoma Mod Pathol. 2016 Mar;29(3):283 doi:10.1038/modpathol.2015.162

14.Huang BP, Lin CS, Wang CJ, Kao SH. Upregulation of heat shock protein 70 and the differential protein expression induced by tumor necrosis factor-alpha enhances migration and inhibits apoptosis of hepatocellular carcinoma cell HepG2. Int. J. Med. Sci. . 2017;14(3):284. doi:  [10.7150/ijms.17861](https://dx.doi.org/10.7150%252Fijms.17861)

15.Matsushima-Nishiwaki R, Toyoda H, Nagasawa T, Yasuda E, Chiba N, Okuda S et.al. Phosphorylated heat shock protein 20 (HSPB6) regulates transforming growth factor-α-induced migration and invasion of hepatocellular carcinoma cells. PloS one. 2016 Apr 5;11(4):e0151907. <https://doi.org/10.1371/journal.pone.0151907>

16.Wang RC, Huang CY, Pan TL, Chen WY, Ho CT, Liu TZ et.al Proteomic characterization of annexin l (ANX1) and heat shock protein 27 (HSP27) as biomarkers for invasive hepatocellular carcinoma cells. PLoS One 2015 Oct 2;10(10):e0139232. <https://doi.org/10.1371/journal.pone.0139232>

17.Gruden G, Carucci P, Lolli V, Cosso L, Dellavalle E, Rolle E et.al. Serum heat shock protein 27 levels in patients with hepatocellular carcinoma. Cell Stress Chaperones. 2013 Mar 1;18(2):235-41. https://doi.org/10.1007/s12192-012-0377-8

18.Van Hees S, Michielsen P, Vanwolleghem T. Circulating predictive and diagnostic biomarkers for hepatitis B virus-associated hepatocellular carcinoma. WJG. 2016 Oct 7;22(37):8271.

doi:  [10.3748/wjg.v22.i37.8271](https://dx.doi.org/10.3748%252Fwjg.v22.i37.8271)

19.Yang Z, Zhuang L, Szatmary P, Wen L, Sun H, Lu Y et.al Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma. Int. J. Med. Sci.  2015;12(3):256. doi:[10.7150/ijms.10735](https://dx.doi.org/10.7150%252Fijms.10735)

20.Zhao YJ, Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. Molecular and clinical oncology. 2013 Jul 1;1(4):593-8. https://doi.org/10.3892/mco.2013.119