Review Article
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Heat shock proteins as diagnostic markers for hepatocellular carcinoma: a novel approach

Abstract

Hepatocellular carcinoma (HCC) is a malignant tumor of the liver. Heat shock proteins play their part in response to cellular stress. They are overexpressed in many forms of cancers where they shield malignant cells from stressful environment thus contributing to poor prognosis and treatment resistance. HSP 27, a heat shock protein mostly associated with Hepatitis induced hepatocellular cancer, inhibits apoptosis and HSP 70 along with HSP 90 contributes to tumor metastasis. HSP 70 count is higher in tumor cells in comparison with normal cells and it is related to tumor size, stage and vessel invasion while HSP 20 count regresses as tumor progresses. Considering all these factors, differentiation of HCC from other liver pathologies becomes much easier at an early stage. Early diagnosis of HCC is important and timely and prompt treatment can decrease the mortality associated with HCC. On the grounds of many useful characteristics like early detection and accuracy in detailing of size, invasion and metastasis of tumor our Review suggests that along with it all HSPs also have therapeutic potential as targeting the specific markers can help patients diagnosed with HCC. HSPs are the innovation from diagnosis to management to cure of HCC. Extensive research exploring Heat Shock Proteins and Hepatocellular Carcinoma was done on search engines such as Science Direct, OVID, Google Scholar, PubMed, and MEDLINE. Articles published in languages other than English were also considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Keywords: Heat-shock proteins, carcinoma, hepatocellular

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Muhammad Asharib Arshad,[1] Haniya Waseem,[2] Mariam Tariq Awana,[3] Hifza Masood[4]

[1]The Children's Hospital and Institute of Child Health, Multan, Pakistan
[2]Victoria Hospital, Bahawalpur, Pakistan
[3]Fatima Memorial Hospital, Lahore, Pakistan
[4]Combined Military Hospital, Multan, Pakistan

Correspondence: Muhammad Asharib Arshad, Department of Dentistry, FMH College of Medicine and Dentistry, Lahore, Tel +92 42 111 555; Pakistan, Email asharibarsha@hotmail.com

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Introduction

Hepatocellular Carcinoma (HCC) accounts for 80% of all carcinomas of liver. This highly prevalent form of cancer is the sixth most common cancer in the world, fifth among the men, eight among women, third in the region of Asia Pacific and primary liver cancer in many countries. The incidence is higher in men, especially above age of 40. The most common cause of HCC in Asia is Hepatitis B and C.[1,2] HCC is a grave health problem because of associated morbidity and mortality. Even after all the advancements made in the healthcare system for its diagnosis and treatment, it's still a major healthcare burden. New effective surveillance and screening programs will decrease morbidity and mortality of patients along with a better life expectancy for people suffering from Hepatitis B and C.

HSPs are a family of highly specialized proteins that play a significant role in 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 management of HCC.

Discussion

HSPs comprise specialized proteins on which extensive research has been done with their relation to Hepatocellular carcinoma. HSPs play an integral role in various stages of the cell cycle. HSPs play a role in the difference processes such as apoptosis, cellular invasion and therapeutic resistance of HCC (Figure 1,Table 1).

Heat shock proteins

Heat shock proteins (HSPs) are also referred to as stress proteins found in all living cells. They play vital role in cell processes such as the cell cycle; signaling pathways; transport, folding and unfolding of proteins and cell protection from apoptosis. This large family of proteins is sub classified according to their molecular weight e.g. HSP 10, HSP 40, HSP 27, HSP 90 etc.

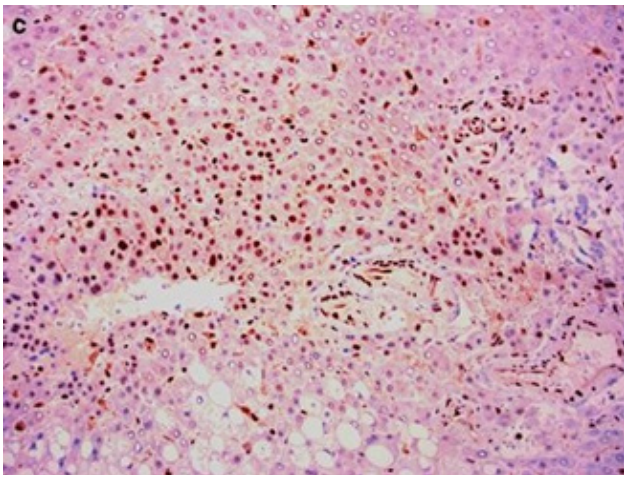

Figure 1 A typical hepatocellular neoplasm in a 57-year old woman with HSP70 that shows nuclear staining (× 200).

[Used after taking permission from; 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 (21)].

Recent studies report that HSPs play role in antigen presentation to class I and II of MHC. They stimulate antigen presenting cells of immune system i.e. macrophages and dendritic cells. Environmental stress also triggers their release in order to control cell damage and promote cellular survival.[4]

Table 1 Role of Heat Shock Proteins in Hepatocellular Carcinoma

HSP	Mechanism	Reference
Apoptosis		
HSP27	Leads to the apoptosis of cells of HCC by inactivating the NF-κB pathway	Guo et al.[32]
HSP90	Integral role in the survival of HCC by regulation of p53 and cyclin D1.	Leng et al.[33]
Therapeutic Resistance		
HSP27	Drugs such as5-fluorouracil and carboplatin become more effective after inhibiting HSP27.	Sharma et al.[34]
HSP90	Bcl-2 inhibitor drugs such as venetoclax become more efficient after inhibiting HSP 90.	Wang et al.[35]
Invasion and Metastasis		
HSP27	Involved in the motility and invasion of HCC cells.	Guo et al.[32]
HSP70	Involved in the migration of HCC cells.	Liu et al.[36]
HSP90	Increase the potential of invasion of HCC cells.	Ma et al.[37]

Abbreviations: HSP, heat shock protein; HCC, hepatocellular carcinoma; Bcl-2, apoptosis regulator Bcl-2; PKC, protein kinase C

HSP overexpression is documented in many cancers as they protect mutated cells from stress in tumor environment and so they are a major factor in poor prognosis as well as treatment resistance faced in different cancers. 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.

The upregulation of heat shock proteins serves as important diagnostic and therapeutic marker. HSP 27 is found in many malignancies and plays vital role in inhibition of apoptosis, RNA splicing, DNA repair, cytoskeletal organization and degradation of oxidized protein.[5] HSP 70 is usually maintained at low levels but is induced under stress conditions including many cancers e.g. breast, colon etc. it also plays crucial role in cellular apoptotic pathway. .[6] HSP 90 is of great significance for being anti-apoptosis and pro-metastasis.[7] Clusterin is also linked to many kinds of cancers and is linked to metastasis and epithelial to mesenchymal transition.[8]

Role of heat shock proteins in hepatocellular carcinoma

HSPs play significant role in HCC and this fact can be used to design specific targeted therapy against them. They have vital role in apoptosis, therapeutic resistance, invasion and metastasis of carcinoma.

Apoptosis

HSP 27 is found in many malignancies. It plays its cytoprotective role by inhibiting caspase-, dependent apoptosis, RNA splicing, DNA repair and degradation of oxidized protein. It also supports cell cycle by inhibiting cytochrome c.[9] In HCC decreased levels of HSP 27 leads to apoptosis of affected cells. Decreased level of HSP 90 are linked with increased levels of p53 resulting in apoptosis. Clusterin protects HCC infected cells from endoplasmic stress-induced apoptosis.

Therapeutic resistance

Therapeutic resistance is the major obstacle in the treatment of HCC. Chemotherapy upregulates HSP 27 and increased levels lead to inhibition of apoptosis.[10] Similar is the case with HSP 90 and clusterin. If we target these biological molecules specifically then resistance can be overcome and therapy against HCC will work more effectively.

Invasion and metastasis

HSP 27 and clusterin overexpression are associated with tumor metastasis, while HSP 70 and 90 are involved in tumor migration.[11]

Diagnostic and prognostic value of heat shock proteins in HCC

HSP 27 exhibits cellular protective response due to its anti-apoptotic properties and increased levels are observed in HCC as well as in chronic Hepatitis B infected cells.

It is actually a chaperon so small siRNA can be used in modulation to produce pro-apoptotic, anti-metastatic and chemo-sensitized effects.OGX-427 is second generation oligonucleotide that targets HSP 27 and suppresses it effectively. .[12] Clusterin, an ATP independent chaperone, being similar to HSP 27 is also targeted by anti-sense oligonucleotide specifically OGX-11 that suppresses clusterin levels promoting apoptosis of infected cells.[13]

HSP 70 is found to be higher in affected cells as compared to normal cells. It serves as a sensitive marker to differentiate HCC from dysplastic nodules, both low and high grade thus leading to early HCC detection. Increased HSP 70 is linked to poor prognosis as well as portal vein and vascular invasion. In addition, HSP 70 along with two other markers glypican-3 and glutamine synthase holds great importance is diagnosing HCC.[14]

17-(Dimethoxy)-17-allylamino geldanamycin (17-AAG) is also reported to prevent ATP binding to HSP 90 via steric inhibition and thus encourages apoptosis.[15]

Outcomes of Clinical trials for Efficacy of Heat Shock Proteins as Tumor Markers

HSP 20

Expression levels of heat shock protein 20 decreases parallel with tumor progression in patients with HCC. Levels of HSP expressed in healthy cells were significantly higher as compared to HCC infected cells.[16] This suggests a suppressive effect on HCC progression. No correlation was found between phosphorylated HSP 27 and HSP 20.

HSP 27

This heat shock protein is mostly associated with hepatitis virus and rate of expression of phosphorylated HSP 27 is higher in HCV antibody positive patients as compared to HBV surface antigen positive patients. Secondly, HSP 27 expression was associated with large tumor size and portal vein invasion.[17] Thus HSP 27 is an overall independent prognostic marker that can determine prognosis and recurrence of HCC.

HSP 70

HSP 70 is associated with tumor size, stage and portal vein invasion. The sensitivity and specificity of HSP 70 was find to be 73.58% and 98.08% respectively. 71.9% of HCC cells were observed to express HSP 70 as compared to 12.1% in nonneoplastic liver cells but it still can't predict overall survival of HCC.[16] When diagnostic value of HSP 70, Glypican 3 and Glutamine synthetase in hepatocellular nodules in cirrhosis was studied. HSP 70 was detected in almost all HCC infected cells. Glypican 3 was found in 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.[14,18] Thus HSP 70 and

27 both can be further investigated to give us better diagnostic and prognostic markers.[19]

Review of current literature

Hepatocellular Carcinoma (HCC) comprises 80% or primary liver cell carcinoma, incidence wise and only 30-40% of diagnosed patients with HCC are at a curable stage.[3] Therefore, the early differentiation, diagnosis and detection of recurrence holds paramount importance in decreasing mortalities from this deadly 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 differentiation of a pre-cancerous lesion from Adenomatous hyperplasia, Atypical Adenomatous hyperplasia and non-cancerous liver lesions.[20]

Also, it differentiates well-differentiated small HCC from High Grade Dysplastic Nodules. .[3] Typical carcinoma was compared to atypical carcinoma and early HCC was compared with advanced HCC and in combination with other markers like p53 and with other biomarkers like GS and Glypican-3 it can help differentiate dysplastic nodules from HCC with sensitivity of 72% and specificity of 100%.[21–24]

Few other forms of HSP help in curing HCC and they are being used as therapeutic targets like HSP 70 increases sensitivity of tumor cells for anti-tumor drugs and help in treating HCC.[25] HSP 20 is also being used for therapeutic purpose in HCC especially in tumors with invasion.[26] Another one is HSP 27 and it alone can tell the overall risks, chances of survival and metastasis in HCC.[3,27] It is found to be elevated in early HCC with positive immunoreactivity of 100% for HCC and levels are particularly related to underlying HCV induced liver cirrhosis.[28] HSF-1 is also being studied as therapeutic target in HCC along with many other HSPs that play role in diagnosis, therapy and management of HCC.[29-30]

According to tumor marker studies for HCC, there are two HSPs, HSP 70 is positively corelated with tumor size, portal vein invasion and tumor stage while HSP 27 was only associated with HCC which is infected by hepatitis virus. In HCC the of HSP 70 and 27 promotes tumor growth and metastasis. Thus, they are potential markers for HCC and should be further investigated.

Furthermore, the expression of HSP 70 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 p53 pathway. In particular HSP 70 has been identified as a potentially sensitive marker to differentiate early HCC from precancerous lesions and 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 a sensitivity of 96% and a specificity of 92% and it also helps in prognosis but usefulness is limited to the 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 and is also good in differentiating HCC from other liver pathology. The only drawback is that it can't effectively detect small tumors as by AFP-L3 and AFP so sensitivity is improved by combining with AFP.[22,31]

Conclusion

Our review suggests that Heat Shock Proteins have bright prospects as the best diagnostic markers for HCC but patient consent must be taken. Apart from studying their therapeutic potential, it will be very beneficial to study them for diagnostic purposes. A major role is played by the HSPs in early detection, differentiation and recurrence of HCC.

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Conflicts of interest

There are no competing interests between the authors.

Authors' contributions

MAA: Conceived and designed the research and wrote the paper.

HW: Did the literature search and assisted in writing the paper.

MT: Revised the manuscript.

HM: Reviewed the manuscript and assisted in writing the manuscript.

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