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hsp70-2 Polymorphism in Urinary Bladder Carcinoma

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Abstract: *hsp70-2 or (hspa1b)* is one of the human *HSP70* genes, it was originally found specifically in primary spermatocytes and spermatids, that played an important role in spermatogenesis. Now *HSP70-2* has attracted increased interest due to its possible involvement in carcinogenesis of non-testicular tissues. *HSP70-2* has been identified as a potential cancer-promoting protein expressed at abnormal levels in a subset of human cancers such as bladder urothelial cancer. So, the present study aimed to study the *HSP70-2* polymorphism in 50 blood sample of patients with urinary bladder carcinoma (UBC) and 15 healthy subjects, also study *HSP70-2* gene expression in 40 paraffin tissue sections of patients with urinary bladder carcinoma UBC and 10 paraffin tissue section in patients with urinary bladder diseases UBD. A PCR-RFLP test was used to study the *HSP70-2* gene polymorphism. r RT-PCR was used to study *HSP70-2* gene expression. Genotype distribution and allele frequencies of *HSP70-2* polymorphisms showed *HSP70-2* P1P2 genotype frequency and *HSP70-2* P2 allele were significantly increased in the patients with UBC compare to healthy subjects($p \leq 0.01$).Also, the expression mean of *HSP70-2* gene is 39.4 fold higher in UBC than in UBD patients.

Key words: Urinary bladder carcinoma , *HSP70-2* polymorphism, PCR.

تعدد طرز الجين المشفر لبروتين الصدمة الحرارية hsp70-2 في سرطان المثانة البولية

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الخلاصة: جين *HSP70-2* هو واحد من بروتينات الصدمة الحرارية البشرية نوع 70 والذي يوجد اصلا في خلايا النطف والخلايا النطفية الأولية اذ يلعب دور مهم في عملية تكوين النطف. وبسبب دوره المتداخل مع تكوين السرطان في انسجة أخرى غير الخصى فقد أصبح حاليا مصدر جذب متزايد وعرف بالبروتين المروج لاحتمالية نمو السرطان حيث يفرز بمستويات غير طبيعية في بعض السرطانات الثانوية مثل سرطان المثانة البولي، لذلك هدفت الدراسة الحالية لدراسة تكرار طرز الجين المشفر لبروتين الصدمة الحرارية *HSP70-2* في 50 عينة دم من مرضى سرطان المثانة و15 عينة دم من أشخاص أصحاء باستخدام تقنية PCR-RFLP، ودراسة التعبير الجيني للبروتين في 40 عينة نسيجية مطمورة في شمع البرافين لمرضى سرطان المثانة البولية و10 عينات نسيجية لمرضى يعانون من أمراض اضطرابات المثانة البولية غير السرطان باستخدام تقنية r RT-PCR . أظهر توزيع النمط الجيني وتكرار الاليل لتشكل بروتين الصدمة الحرارية *HSP70-2* فرق معنوي عالي لتكرار النمط الجيني نوع P1P2 والاليل نوع P2 في مرضى سرطان المثانة مقارنة بالأشخاص الأصحاء، بنسبة احتمالية ($p \leq 0.01$) . وكان معدل التعبير الجيني أعلى بنسبة (39.4) مرة في مرضى سرطان المثانة مقارنة بمرضى اضطرابات المثانة.

Introduction

HSP70-2 or (HspA1B) is one of the human HSP70 genes , it encodes a heat-inducible protein HSP70. The gene is polymorphic, potentially accounting for variation in its functions and susceptibility to stress tolerance [1]. An adenine (A) to guanine (G) polymorphism at HspA1B +1267 has been described; however, the polymorphism does not lead to any amino acid change in the HSP70 protein. Carriage of the G allele is associated with increased risks and poor outcomes in different diseases [2],[3] & [4].

HSP70-2 was originally found specifically in primary spermatocytes and spermatids, described as a testis-specific protein that played an important role in spermatogenesis [5]. Aberrant expression of HSP70-2 in testes induced primary spermatocytes to arrest in meiosis I and undergo apoptosis, which was leading to male infertility. Now HSP70-2 has attracted increased interest due to its possible involvement in carcinogenesis of non-testicular tissues. HSP70-2 has been identified as a potential cancer-promoting protein expressed at abnormal levels in a subset of human cancers, such as, breast cancer [6], bladder urothelial cancer [7] , esophageal squamous carcinoma and malignant tumors[5] . Some levels of the HSP70-2 gene activity was also observed in cell lines derived from several human cancers[8], while silencing of the HSP70-2 gene in cancer cells led to growth arrest and decrease in tumorigenic potential [7],[9] . A HSP70-2 mutation was recognized by cytotoxic T lymphocyte (CTL) on a human renal cell carcinoma Overexpression of HSP70-2 is correlated with increased cell proliferation, poor differentiation and lymph node metastases in human breast cancer, cervical cancer and bladder urothelial cancer [5 ,7]. The highest level of HSP70-2 was also detected in cells of the basal layers of the skin, esophagus and bronchus epithelia.

Depletion of HSP70-2 in cancer cells induces G1 arrest and senescence that are mediated by an up-regulation of the expression of macrophage inhibitory cytokine-1 (MIC-1). Interestingly, cancer cells depleted for HSP70-1 and HSP70-2 display strikingly different morphologies, cell cycle distributions, and gene expression profiles, indicating that in spite of their remarkable homology, HSP70 family members either display specificity for their client proteins or serve chaperone-independent specific functions in cancer cells [10] .Most normal cells respond to death stimuli by undergoing caspase-dependent apoptosis. In contrast, cancer cells frequently escape spontaneous and therapy-induced caspase activation due to acquired mutations in their apoptotic machinery [11],[12] .

Thus, the aim of the present work was to study the frequency of polymorphism PstI site of Hsp70-2 gene SNP typing in Iraqi patients with urinary bladder carcinoma UBC as a risk factor to development it , and evaluate HSP70-2 gene expression of them.

Materials and Methods

The period of the study from May-2011 to May-2012 were eligible for this study. The cases were diagnosed clinically by consultant urologists at Al-Yarmook Teaching Hospital, and Baghdad Hospital for Specialists Surgeons .

Ethical permission to conduct the research was obtained from these hospitals and from all participants in this study. Selections of the patients were accomplished with the assistance of surgeons in the hospitals.

1-DNA Extraction and HSP70-2 Polymorphism

DNA was extracted from whole blood samples of each patient (50) and healthy control (15) by using The ReliaPrep™ Blood gDNA Miniprep System (promega,USA) .The coding sequence of the hsp70-2 gene was amplified from genomic DNA by using sequence specific oligonucleotide primers: 5¯CATCGACTTCTACACGTCCA-3¯ and antisense (nucleotide 2180–2199 within the 3¯ untranslated end to avoid HSP70-1 homology) 5¯-CAAAGTCCTTGAGTCCCAAC-3¯. The reaction mix and PCR conditions are given in (Table 1) and (Table 2). The PCR product was subjected to electrophoresis on 2% agarose gel ,the PCR product band was 1117 bp.

Table (1): The PCR reaction mix (25 µl) for HSP70-2 gene.

Chemicals	Volume
DNA	5 µl (25 ng)
Hot start master mix	12.5 µl
Primer forward	1 µl
Primer reverse	1 µl
Nuclease free water	5.5 µl
Total volume	25 µl

Table (2): PCR conditions for HSP 70-2 genes.

Steps	Temperature	Time	No. of cycles
Denaturation 1	94°C	3 min.	1
Denaturation 2	94°C	1 min.	35
Annealing	61 °C	1min	
Extension 1	72°C	2 min.	
Extension 2	72°C	10 min.	1

Image for the gel were captured by Gel Documentation s(140 system) imaging system provided by votornix /USA. Then, the images were printed.

The corresponding PCR products were digested with PstI restriction enzyme to assess the polymorphism of the HSP70-2 at position 1267. The digestive reaction mix include :

RE 10 X buffer 2 µl
Acetylated BSA,10 µg/ µl 0.2 µl
DNA,1 µg/ µl 1 µ
Nuclease free water 16.3 µl
Restriction enzyme ,10 µl 0.5 µl

The final volume 20 µl was mixed gently by pipetting ,and centrifuged for a few seconds , then incubated at the optimum temperature for overnight .

The incubated samples were subjected to electrophoresis on 2% Agarose gel to characteristic yielded fragments patterns .

The two allelic forms (P1and P2) of HSP70-2, corresponding to the presence or the absence of the PstI site, are referred to as HspP1 and HspP2, respectively .The presence of a PstI site was indicated by the cleavage of the 1117 bp amplified product to yield two fragments of 936 and 181 bp (P1) while the absence of a PstI site was indicated by resulting a single fragment which was 1117 bp in size . In homozygote individual both alleles show absence or present of the PstI restriction site .However, in heterozygote individual one allele shows the presence of the PstI restriction site while the other shows the absence of the PstI restriction site . So the presence of the three fragments (1117 bp, 936 and 181 bp) indicates the heterozygote allelic form P1P2.

2-RNA Extraction from FFPE Tissues and HSP70-2 Gene Expression

This part of the study was performed at Institute of Liver Studies in King's College Hospital /London /UK.

Total RNA was isolated from 50 fixed formaline paraffin -embedded tissue sections (FFPE) (40 UBC and 10 UBD (control)) by using RNeasy FFPE kit (Qiagen, West Sussex, UK) according to the manufacturer protocol. Omniscript® Reverse Transcriptase kit (Qiagen, West Sussex, UK) was used to generate complementary DNA (cDNA) for twenty three only of total RNA samples that were given a good absorbance and quality .

Gene expression was evaluated in the presence of endogenous control 18s rRNA . All samples were analysed in triplicates. The TaqMan® gene expression assay for HSP70-2 was used and 18s rRNA (Applied Biosystems, Europe B.V. UK branch, Warrington, UK). The rRT-PCR reaction mixture (20µl) per well of a 96-well ABI Prism consisted of the following Table (3):

Table (3) : The r RT-PCR reaction mixture.

Chemicals	Volume
20x gene expression assay (probe)	1 µl
2x TaqMan® Universal Master Mix	10µl
cDNA	2µl
Sterile water	7µl
Total volume	20 µl

For negative control, cDNA was replaced with an equivalent volume of sterile water, i.e. no-template control (NTC).

Results and Discussion

The polymorphism of HSP70-2 at position 1267 associates with severity of different diseases, like, for example, Crohn's disease [3] and acute pancreatitis [2] , also it has been suggested to be associated with carcinogenesis in many malignant cancer tissues like bladder urothelial cancer and esophageal squamous carcinoma [7],[5] . Our study is the first, investigating association between HSP70-2 polymorphism genotypes and UBC in Iraqi patients .

Genomic DNA was isolated from blood cells, all samples yielded intact genomic DNA as shown in (Figure 1) . In this study , DNA was isolated from blood sample of patients and healthy control groups.

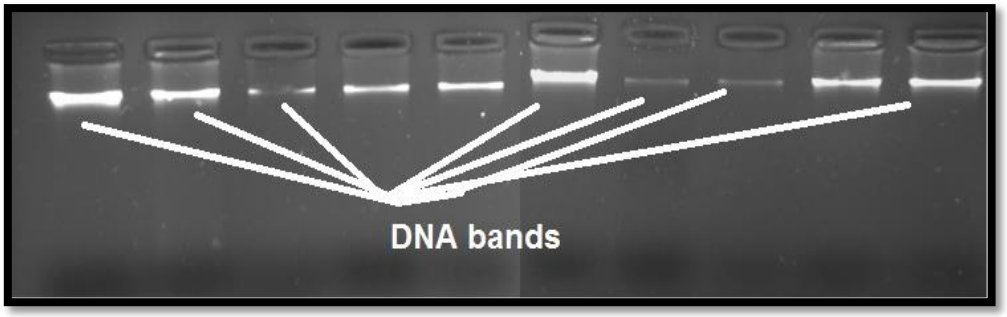


Figure (1): Purity of genomic DNA band on 0.8% agarose gel at 80 voltage for one hour .

PCR analysis was accomplished for the 50 samples of UBC patients and 15 healthy control. HSP70-2 was detected by characteristic band patterns on 2% Agarose .The PCR product was 1117bp , (Figure 2). The Pst I digest of HSP70-2 PCR product that incubated overnight with Pst I restriction enzyme at optimum temperature was detected to characteristic yielded fragments patterns on 2% Agarose.

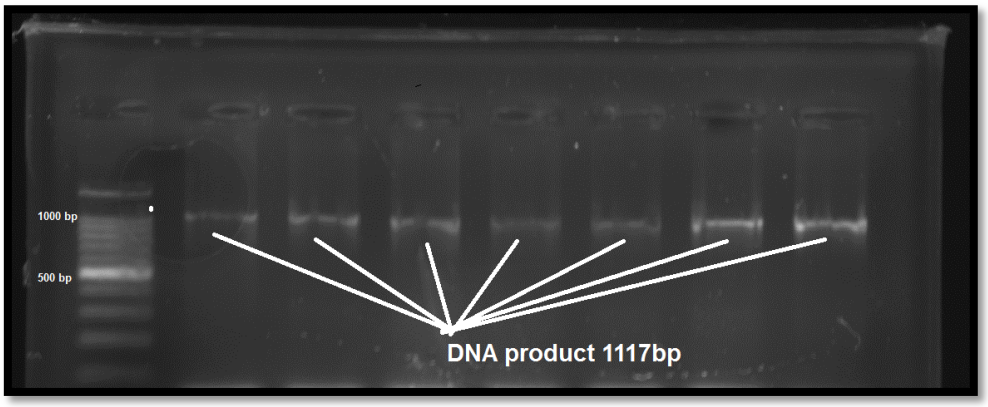


Figure (2) : PCR product for HSP70-2 (1117 bp) on 2% agarose gel at 80 voltage for one hour.

Genotype distribution and allele frequencies of HSP70-2 polymorphisms are shown in (Table 3) and(Table 4), There was a high significant difference among frequencies HSP70-2 genotypes polymorphism with study groups(UBC and healthy controls , p≤ .01) . Thus ,HSP70-2 P1P2 genotype frequency was significantly increased in the patients with UBC (p≤0.01) . Also, there was a high significant difference among frequency of HSP70-2 alleles with study groups(p ≤ 0.01), HSP70-2 (P2) allele occurred more frequently in UBC than in healthy subjects . From the above results ,we found that HSP70-2(P2) allele and (P1P2) polymorphism genotype was associated with a higher risk UBC.

These results are in agreement with the study by Jeng et al. (2008) in which the authors demonstrated that the frequencies of the HSP70-2 P2 allele in HCC patients were higher than in unrelated controls (p = 0.0001) [13].

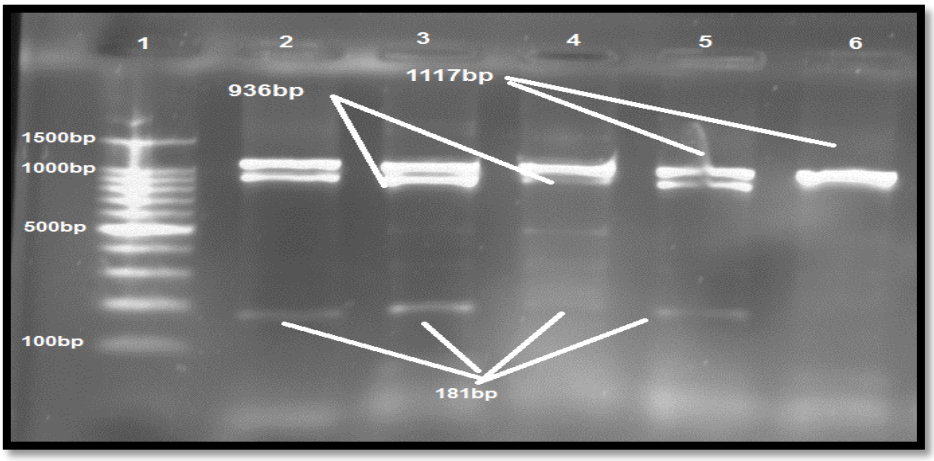
Comparison of HSP70-2 genotype frequencies in patients with UBC and healthy indicated absence of P1P1 genotypes in both groups resulting in a decrease of the P1 allele in them ,this absence may be because the small number of study samples , so we didn't have a chance to show it ,these results agreed with El-Din et al. (2010) who found that the A/A genotype frequency was 0% in hepatocellular carcinoma (HCC) Egyptians patients [14]. The Pst I digest of HSP70-2 PCR product is shown in (Figure 3). Individual in line 6 was homozygous for the absence of the pst I restriction site (P2 P2) resulting in a single fragment which was 1117 bp in size . Individuals in lines 2,3.4,5 were heterozygous for the pst I restriction site (P1 P2) resulting three fragments, two for P1 allel (936 bp the larger fragment in size, while the smaller fragment was 181 bp) in addition to one fragment 1117 bp in size belong to the P2 allele.

Table (3): Genotypes distribution of HSP70-2 polymorphism among UBC patients and healthy control.

HSP70-2	UBC	Healthy	Total NO.
P1 P1	0/50(0%)	0/15(0%)	0/65(0.0%)
P2 P2	19/50(38%)	9/15(60%)	28/65(43.1%)
P1 P2	31/50(62%)	6/15(40%)	37/65(56.9%)
Total NO.	50/65(76.9%)	15/65(23.1%)	65/65(100%)
P-value $\chi 2$ –value	0.0025 9.425 **	0.0039 9.013 **	0.0027 9.250 **

Table (4): Alleles frequencies of HSP70-2 polymorphisms in UBC patients and healthy control.

HSP70-2 (allele frequency)	UBC No..	Healthy No.	Total NO.
P1	31/100(31%)	6/30(20%)	37/102(36.3%)
P2	69/100(69%)	24/30(80%)	93/102(63.7%)
Total NO.	100/130(76.9%)	30/130(23.1%)	130(100)
P-value $\chi 2$ –value	0.0062 7.565 **	0.0001 10.500 **	0.0082 7.178 **



Figure(3) :HSp70- 2 (pst I) polymorphisms:1,DNA ladder (100 bp) ,2,3,4,and 5 Individuals were heterozygous for the pst I restriction site (P1 P2; 1117 bp, 936 bp and 181bp). 6,Individual was homozygous for the absence of the pst I restriction site (P2 P2) resulting in a single fragment (1117 bp).

In Iraq , the allele and genotype frequencies of HSP70-2 were not studied , and in this study the association between the polymorphism PstI site of HSP70-2 gene and UBC had been shown for the first time.

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HSP70-2 Gene Expression

This part of the study was performed at Institute of Liver Studies in King's College Hospital /London /UK . We successfully isolated total RNA from 23 out of 50 samples ,these 23 samples showed a good quality and absorbance when measured their quantity by nano-spectrophotomoter after that we used these sample to prepare c DNA that used in rRT-PCR . Other samples (27) showed bad results based on absorbance and its quality that reflects extensive degradation for tissue , this may belong to the long period of storage, condition of storage and kind of paraffin . In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level means the greater amount of target nucleic acid in the sample). In this study, the cycle threshold (Ct) values were obtained from the samples (Figure 4) . For each sample, the difference between HSP70-2 and 18s rRNA was already calculated by the software.

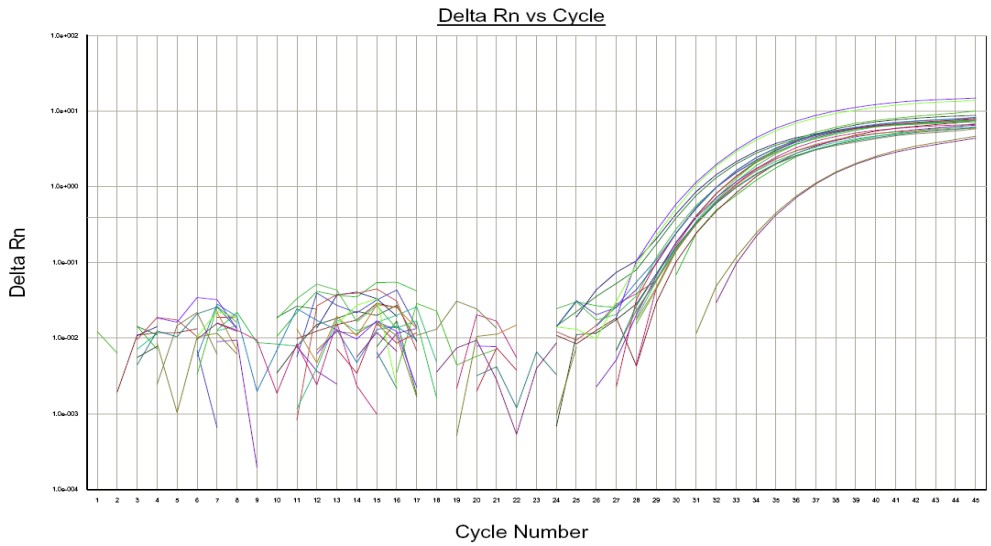

Figure (4): Amplification of HSP70-2 ,18srRNA product run with triplicate samples, cycle number is plotted on the X-axis with level of fluorescence on Y-axis .The threshold fluorescence level depicted by green line.

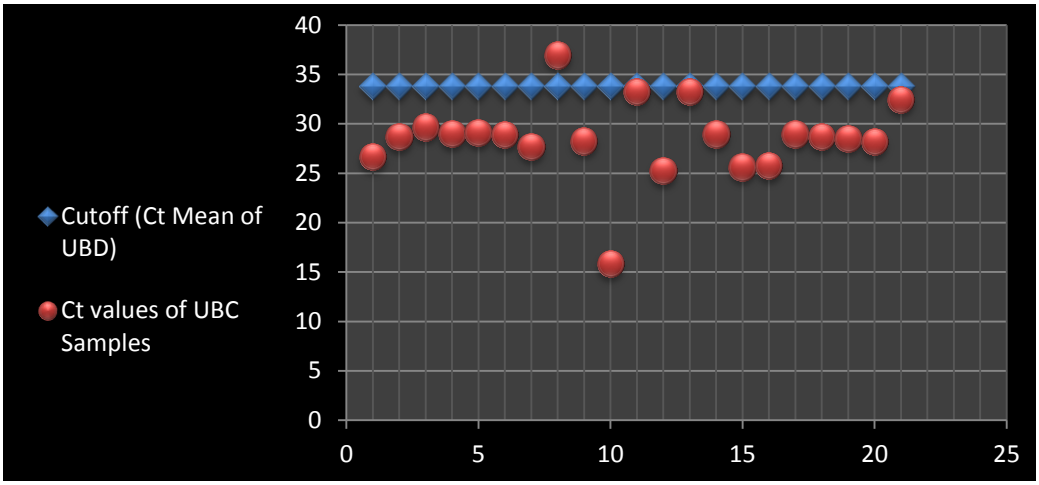

Figure (5): The relative expression of the HSP70-2 gene UBC patients compare to HSP70-2 expression mean of UBD as calibrator.

The highest Ct means value were observed in urinary bladder disorders patients (33.8) with comparison to urinary bladder carcinoma (28.5), and that means UBC showed a high abundance of the HSP70-2 gene/ mRNA (increase gene expression) more than UBD , the difference between the Ct for UBC patient and Ct for UBD patient control sample is the "delta Ct" which was used to find calculating the gene expression fold . A mean value of Ct in UBD patients(control) used as calibrator to normalization against unit of mass used (HSP70-2 gene), the expression of the HSP70-2 gene in all other samples of UBC patients is expressed into high and low mRNA-expressing samples relative to calibrator which showed high statistically significant differences between high mRNA-expressing samples with low Ct in 20 patients (80.96%) and low mRNA-expressing samples with high Ct in only one patient (19.04%, $P \leq 0.01$) (Figure 5).

Normalized equation simplifies to:

Ratio (test/calibrator) =2(Ct calibrator-Ct test) .

Also the relative expression mean of HSP70-2 gene in UBC and UBD patients was:

Ratio (UBC/UBD) =2(Ct UBD-Ct UBC) =2(33.8-28.5) =2(5.3) =39.4

The expression mean of HSP70-2 gene is 39.4 fold higher in UBC than in UBD patients .

Our results agreed with Zhang et al. [5] who found that the expression levels of HSP70-2 mRNA and protein in the esophageal squamous cell carcinoma (ESCC) tissues were significantly higher than in non-cancerous tissues and normal.

As a chaperone protein, HSP70-2 is essential for the growth of spermatocytes and cancer cells, and it is known to be involved in apoptosis and regulation of cell proliferation

[16]. However, the underlying mechanisms for the high expression of HSP70-2 in tumors remain incompletely understood, but likely involve regulatory processes, such as cell cycling. Indeed, HSP70-2 appears to be a molecular chaperone for CDC2 and is required for CDC2/cyclinB1 complex formation, whose destruction can prevent development of the CDC2 kinase activity, required to trigger G2/M phase transition [17] . HSP70-2(HSPA2) overexpression protects V79 fibroblasts against bortezomib-induced apoptosis [18].The mechanism of a mutated HSP70-2(HSPA2) chaperone has a dominant effect in tumor cells by triggering the G2/M phase transition during the mitotic cell cycle, which could explain the HSPA2 abnormal expression in somatic tumors. HSPA2 may also have a potential role in cancer pathogenesis by participating in the regulation of antitumor immunity, such as acting as a chaperone molecule for immunogenic tumor associated peptides[19], while HSPA2 has been identified as a putative susceptibility locus in organ-specific autoimmune diseases. Lastly, a new regulatory mechanism of HSPA2 expression in tumor cells has been disclosed, which suggests that the upregulation of HSPA2 enhanced the resistance of tumor cells to hypoxia-induced apoptosis, which provides a new insight into how tumor cells overcome hypoxic stress and survive . It is well known that the HSP70-2 is localized primarily in cytoplasm at physiological temperature whereas HSP70-2 migrates to the nucleus and nucleoli under heat-shock conditions [20].The phenomenon of HSP70-2 translocation into the nucleus and nucleoli has been presently found in many other heat-shock cancer cells, which is part of a cellular protective response under stress conditions, but the mechanism is currently unknown. Generally, HSP70-2 is involved in intracellular trafficking and nuclear receptor binding, which also prevents inappropriate protein aggregation and mediates transport of immature (or damaged) proteins to target subcellular compartments for final packaging, degradation or repair [21]. Since HSPA2 shuttles continuously between the cytoplasm and nucleus during heat-shock, this cell migration is critical for tumor formation and metastasis.

Conclusions

There was a significant high frequently in HSP70-2 P1P2 genotype and HSP70-2 P2 allele of patients with UBC that have led to be as a risk factor. Differences in the gene expression levels of HSP70-2 in UBC compared with UBD have led to HSP70-2 being investigated as diagnostic and prognostic biomarker in bladder cancer.

References

1. Zhou F, Wang F, and Li F (2005) Association of hsp70-2 and hsp-hom gene polymorphisms with risk of acute high-altitude illness in a Chinese population. Cell Stress Chaperones; 10(4):349–356.
2. Balog, A. , Gyulai, Z. , Borom, L.G., Farkas, G., Takacs T, Lonovics J, and Mandi Y (2005) Polymorphism of the TNF-alpha, HSP70-2, and CD14 genes increases susceptibility to severe acute pancreatitis. Pancreas; 30(2): 46–50 .
3. Klausz G, Molnar T., Nagy F., Gyulai Z., Boda K, Lonovics J., and Mandi Y. (2005). Polymorphism of the heat-shock protein gene Hsp70-2, but not polymorphisms of the IL-10 and CD14 genes, is associated with the outcome of Crohn's disease. Scand J Gastroenterol 40 (10):1197–1204.
4. Giacconi R, Cipriano C, Muti E Costarelli L, Malavolta M, Caruso C, Lio D, and Mocchegiani E (2006) .Involvement of -308 TNF-alpha and 1267 Hsp70-2 polymorphisms and zinc status in the susceptibility of coronary artery disease (CAD) in old patients. Biogerontology 7(5-6):347–356.
5. Zhang H., Chen W., Duan C., and Zhang C. .(2013). Overexpression of HSPA2 is correlated with poor prognosis in esophageal squamous cell carcinoma. World Journal of Surgical Oncology, 11:141.
6. Mestiri, S, Bouaouina N, Ahmed SB, Khedhaier A, Jrad BB, Remadi S, and Chouchane L: (2001) . Genetic variation in the tumor necrosis factor-alpha promoter region and in the stress protein hsp70-2: susceptibility and prognostic implications in breast carcinoma. Cancer, 91(4):672–678.
7. Garg M., Kanojia D., Seth A, Kumar R, Gupta A, Surolia A, and Suri A: (2010). Heat-shock protein 70–2 (HSP70-2) expression in bladder urothelial carcinoma is associated with tumour progression and promotes migration and invasion. Eur J Cancer, 46(1):207–215.
8. Piglowski W, Nowak R, Krawczyk Z, and Scieglinska D: (2007).The structural and functional analysis of the human HSPA2 gene promoter region. Acta Biochim Pol, 54:99–106.
9. Xia LM, Tian DA, Zhang Q, Yan W, Wang B, Liu M, and Li PY, B C:(2008) . Inhibition of HSP70-2 expression by RNA interference induces apoptosis of human hepatocellular carcinoma cells. Chinese J. of Hepatology , 16(9):678–682.
10. Rohde M, Daugaard M, Jensen MH, Helin K, Nylandsted J, and Ja¨a¨ttela¨ M. (2005).Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. Genes Development.19 (5):570–582.
11. Hanahan D,and Weinberg RA. (2000).The hallmarks of cancer. Cell. 100(1):57–70.
12. Jaattela ¨ M. (2004).Multiple cell death pathways as regulators of tumour initiation and progression . Oncogene;23(16): 2746–2756.
13. Jeng J.E, Tsai J.F, Chuang LY, Ho MS, Lin ZY, Hsieh MY, Chen SC, Chang WL, Wang LY, Yu ML, Dai CY, and Chang JG (2008). Heat shock protein A1B 1267 polymorphism is highly associated with risk and prognosis of hepatocellular carcinoma: a case-control study. Medicine (Baltimore) ;87(2):87-98.
14. El-Din HG, Ghafar NA, Saad NE, Aziz M, Rasheed D, and Hassan EM. (2010).Relationship between codon 249 mutation in exon 7 of p53 gene and diagnosis of hepatocellular carcinoma. Arch Med Sci;6(3):348-55.
15. Rehman S. U., Sameer A. S., Zahoor L., Syeed N., Nanda M. S., Hafiz A., Shah Z. A., and Siddiqi M. A (2009).Polymorphic analysis of MHC linked Heat Shock Protein 70 genes: Their susceptibility and prognostic implication in Kangri cancer cases of Kashmiri population Indian. J Hum Genet.15(2): 65–71.
16. Daugaard M, Jaattela M, and Rohde M: (2005). Hsp70-2 is required for tumor cell growth and survival. Cell Cycle, 4:877–880.
17. Zhu D, Dix DJ, and Eddy EM: (1997).HSP70-2 is required for CDC2 kinase. activity in meiosis I of mouse spermatocytes. Development, 124(15):3007–3014
18. Filipczak PT, Piglowski W, Glowala-Kosinska M, Krawczyk Z, and Scieglinska D: (2012) . HSPA2 overexpression protects V79 fibroblasts against bortezomibinduced apoptosis. Biochem Cell Biol, 90:224–231.

19. Feng H., Zeng Y, Graner, M.W., Likhacheva A, and Katsanis E: (2003). Exogenous stress proteins enhance the immunogenicity of apoptotic tumor cells and stimulate antitumor immunity. Blood, 101:245–252.

20. Scieglinska D, Piglowski W, Chekan M, Mazurek A,and Krawczyk Z: (2011). Differential expression of HSPA1 and HSPA2 proteins in human tissues; tissue microarray-based immunohistochemical study. Histochem Cell Biol, 135:337–350.

21. Widlak W, Vydra N, Malusecka E, Dudaladava V, Winiarski B, Scieglinska D, and Widlak P: (2007).Heat shock transcription factor 1 down-regulates spermatocyte-specific 70 kDa heat shock protein expression prior to the induction of apoptosis in mouse testes. Genes Cells, 12:487–499.