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Heat-shock protein 70-2 (HSP70-2) expression in bladder urothelial carcinoma is associated with tumour progression and promotes migration and invasion

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ABSTRACT

Purpose: Testis specific heat-shock protein 70-2 (HSP70-2), a member of HSP70 chaperone family, is essential for the growth of spermatocytes and cancer cells. We investigated the association of HSP70-2 expression with clinical behaviour and progression of urothelial carcinoma of bladder.

Experimental design: We assessed the *HSP70-2* expression by RT-PCR and HSP70-2 protein expression by immunofluorescence, flow cytometry, immunohistochemistry and Western blotting in urothelial carcinoma patient specimens and HTB-1, UMUC-3, HTB-9, HTB-2 and normal human urothelial cell lines. Further, to investigate the role of HSP70-2 in bladder tumour development, HSP70-2 was silenced in the high-grade invasive HTB-1 and UMUC-3 cells. The malignant properties of urothelial carcinoma cells were examined using colony formation, migration assay, invasion assay *in vitro* and tumour growth *in vivo*.

Results: Our RT-PCR analysis and immunohistochemistry analysis revealed that HSP70-2 was expressed in both moderate to well-differentiated and high-grade invasive urothelial carcinoma cell lines studied and not in normal human urothelial cells. In consistence with these results, HSP70-2 expression was also observed in superficially invasive (70%) and muscle-invasive (90%) patient's tumours. Furthermore, HSP70-2 knockdown significantly suppressed cellular motility and invasion ability. An *in vivo* xenograft study showed that inhibition of HSP70-2 significantly suppressed tumour growth.

Conclusions: In conclusion, our data suggest that the HSP70-2 expression is associated with early spread and progression of urothelial carcinoma of bladder cancer and that HSP70-2 can be the potential therapeutic target for bladder urothelial carcinoma.

1. Introduction

Bladder cancer is the second most common malignancy of the genitourinary tract and the fourth major cause of death among men.[1] Although radical cystectomy is considered as the 'gold standard' for treatment of patients with localised but muscle-invasive bladder cancer, about 50% of such patients develop metastases within 2 years after cystectomy.[2]

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Urothelial carcinoma of the bladder accounts for more than 90% of bladder cancers.[3] Most urothelial carcinomas (~80%) present as superficially invasive tumours, which include Ta (non-invasive) or T1 (lamina propria invasive) tumour. Muscle-invasive cancer (T2–4) has a much less favourable prognosis than superficial cancer, despite aggressive multimodal therapy.[4] Due to the unfavourable prognosis of muscle-invasive cancer, there is a need for developing markers that can identify superficial cancers with a high risk of progression. The characterisation of such type of marker will help to detect the life-threatening invasive bladder cancer and thus improve the prognosis of this disease.

Heat-shock proteins (HSPS) are a group of evolutionarily highly conserved chaperone proteins induced by a variety of environmental and pathophysiological stresses[5] and are over-expressed in a wide range of human cancers. The human HSP70 family has at least eight homologous proteins having different cellular localisation and expression pattern.[6] Among this protein family, HSP70 is the major stress-inducible protein that is abundantly and ubiquitously expressed in all cells. HSP70 expression has been reported in malignant tumours of various origins.[7] In addition various studies have demonstrated the tumourigenic potential of HSP70 in rodents.[8,9] Furthermore, the ablation of HSP70 protein revealed inhibition in cancer cell growth not only in the cell culture but also in tumour xenograft in mice.[10,11] In contrast, other HSP70 chaperone family proteins such as HSP70t (HSP70-Hom or HSP701L) and HSP70-2 (HSPA2) are abundantly expressed in testis and shows rare or no expression in other tissues.[12,13] Especially, HSP70-2 has been reported to play an important functional role in the testis and is involved in the first meiotic division of male germ cells and growth of spermatocytes during spermatogenesis.[14] *HSP70-2* gene is mapped to human chromosome 14q24, a region implicated in colorectal cancer and is involved in amplification and expression of colorectal cancer-related genes.[12] Recently, HSP70-2 expression was also demonstrated in a subset of primary and metastatic breast cancer specimens.[6] The process of carcinogenesis involves a complex array of genetic and epigenetic alterations, which may contribute to cancer pathogenesis.[15] Moreover, the association and molecular mechanisms for HSP70-2 expression in cancer cells, early spread of cancer and metastasis remain unclear. Therefore, further studies are warranted to understand whether altered expression of the HSPs at gene or protein level is of importance to cancer risk assessment, diagnosis, prognosis and cancer treatment for better cancer management.

Recent studies on gene microarray expression analysis of HeLa cervix carcinoma cells have reported HSP70-2 gene expression along with other HSP70 genes.[6] In the present study, we report the expression of HSP70-2 in superficially invasive and muscle-invasive urothelial carcinoma of bladder cancer patients, correlating its expression with the pathological and clinical data. We further evaluated its potential role and provide evidence for its association in cell migration, invasion and tumour growth in urothelial carcinoma cells.

2. Materials and methods

2.1. Tissue specimens and cell lines

A total of 118 urothelial carcinoma of bladder patients tissues (male: 98; female: 20) and 45 matched adjacent non-cancerous tissue (ANCT) specimens were obtained from Department of Urology, All India Institute of Medical Sciences, New Delhi, India, in accordance with local Ethics Committee. Tissues were obtained after patient's written consent; 60 patient's specimens who underwent TURBT for the treatment of Ta, Tis and/or T1; and 58 patients who underwent radical cystectomy for the treatment of T2, T3 and/or T4. Pathologic reports were provided by the organisation for tissue. The 2004 WHO bladder tumour classification criteria (low grade and high grade) were used for grading[16] and pathologic staging was done according to the 2002 tumour-lymph node-metastasis classification system.[17] Detailed clinical characteristics of the patients are listed in Table 1. Four human urothelial carcinoma cells, HTB-2 (RT-4), HTB-9 (5637), HTB-1 (J82) and UMUC-3 cells were purchased from the American Type Culture Collection (Rockville, MD) and cultured in minimal essential medium (MEM) supplemented with 10% heat-inactivated foetal calf serum (FCS) and 50 mg/ml Gentamycin, 100 mg/ml Streptomycin (Invitrogen, Carlsbad, CA) under standard conditions. Normal human urothelial (NHU) cell line was established (18–35 passages *in vitro*) from surgical specimens from patients with no history of bladder cancer and maintained as earlier described.[18]

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from frozen bladder urothelial carcinoma patient's tissues and urothelial carcinoma cells using the TRI Reagent (Ambion Inc., Austin, TX) according to the manufacturer's protocol as described earlier.[19] RT-PCR was performed as described earlier.[20]

2.3. HSP70-2 antibodies, immunofluorescence microscopy, flow cytometric, Western blot analysis and immunohistochemistry

The primary antibodies used included murine monoclonal antibodies against HSP70 and HSC70 (C92FBA-5 and N27F3-4, Calbiochem, San Diego, CA). Antibodies against HSP70-2 were generated by immunising rabbit with ovalbumin-conjugated NH2-SKLYQGGPGGGGSSGPGPT peptide corresponding to the amino acids 611–628 in the published *HSP70-2* sequence (Accession No.: NM_021979; Gene symbol HASPA2). HSP70-2 expression in urothelial carcinoma cells was confirmed by indirect immunofluorescence,[21] flow cytometric and Western blot analysis as previously described.[19]

Paraffin-embedded sections (4 μm) of urothelial carcinoma specimens, and ANCT were analysed for HSP70-2 protein expression using anti-HSP70-2 antibody as described earlier.[19] We determined the HSP70-2 immunostained cells by counting >500 cells from 5 random fields of each

Table 1 – HSP70-2 expression (RT-PCR/IHC-IRS) and clinicopathological characteristics of bladder urothelial carcinoma.

Pathologic and clinical features	HSP70-2 expression	
	RT-PCR/IHC (positive/tested)	IHC-IRSs Means ± SE
Age (years)		
Mean (range)	57 (18–95)	
Adjacent non-cancerous tissue (ANCT)	0/45 (0%)	
All tumours	94/118 (80%)	68.63 ± 1.39
Pathologic tumour stage		
Ta	9/18 (50%)	67.00 ± 5.16
Tis	10/10 (100%)	61.00 ± 3.40
T1	23/32 (72%)	60.38 ± 3.29
T2	22/25 (88%)	74.30 ± 1.96
T3	18/21 (86%)	72.00 ± 2.26
T4	12/12 (100%)	76.00 ± 3.36
Superficially invasive tumours (Ta + Tis + T1)	42/60 (70%)	61.98 ± 2.24
Muscle-invasive tumours (T2 + T3 + T4)	52/58 (90%)	74.02 ± 1.37
Pathologic tumour grade		
Low grade	43/60 (72%)	60.58 ± 2.10
High grade	51/58 (88%)	75.43 ± 1.23
Statistical analysis (P values of different test used in this study)		
Clinicopathological features	Student's *t*-test IHC-IRS	Pearson χ^2-test RT-PCR/IHC
Superficial and invasive	<0.0001	0.008
Stages Ta, Tis and T1	–	0.021
Stages T2, T3, and T4	–	0.405
Low and high grades	<0.0001	0.028

specimen under ×400 magnification in each tissue section as described earlier.[22] Immunoreactivity score (IRS) was designated as the percentage of cancer cell showing immunoreactivity against HSP70-2 expressed protein. We considered a distinct positive immunoreactivity in a specimen showing >10% of cancer cells stained for HSP70-2 protein.

2.4. *siRNA knockdown of HSP70-2 gene expression*

Four HSP70-2-specific shRNA (SureSilencing shRNA plasmids) and control shRNA vector were purchased from SuperArray (Frederick, MD, USA). The targeted sequences were shRNA1: CAT AAC GGT CCC GGC CTA TT; shRNA2: GAG CGG TAC AAA TCG GAA GAT; shRNA3: CGG CGA CAA ATC AGA GAA TGT; and shRNA4 TTC GAC GCC AAG AGG CTG ATT. HSP70-2shRNA design was based on the GenBank Accession No.: NM_021979; Gene symbol HASPA2. Negative control NC: GGAATCTCATTCGATGCATAC. HSP70-2shRNA transfection was performed in opti-MEM with the transfection reagent LipofectAMINE Plus (Invitrogen Life Technologies) following the manufacturer's instructions.

To examine the biological role of HSP70-2 in tumourigenesis, *in vitro* cellular growth, colony formation, cell invasion, migration and wound healing assays were carried out as described earlier.[19] For *in vivo* studies, athymic nude mice were maintained in pathogen-free conditions in our animal facility. Athymic nude mice [Nii:NIH (S; nu/nu)] at 5 weeks of age were inoculated subcutaneously (s.c.) with 1×10^7 cells (HTB-1) mixed with matrigel (1:1) in the right flank to initiate tumour growth. Tumour volume was measured with calipers and calculated using the following formula: $\pi/6 \times (\text{longest diameter}) \times (\text{shortest diameter})^2$. When the tumour volume reached around 50–100 mm^3, animals were randomly divided into experimental and control groups ($n = 6$) and were intratumourally injected with HSP70-2shRNA3 (50 μg) or NCshRNA plasmids (50 μg). To facilitate delivery of the vectors *in vivo*, the liposome transfection reagent LipofectAMINE Plus was applied in the ratio as follows: vector (μg)/Lipo (vol)/OPTI (vol) = 1 μg:3 μl:100 μl. Intra-tumour injection was performed every 3 d, for 6 weeks and tumour growth was evaluated by tumour volume every 3 d for 7 weeks. At the end of the treatment, all mice were sacrificed, and the tumours were removed, weighed and fixed in 10% buffered formalin for HSP70-2 expression by immunohistochemistry as described earlier.[22]

2.5. *Statistical analysis*

The Pearson χ^2-test and unpaired Student's *t*-test were performed using SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL). Results were expressed as the means ± SE. All *P* values were 2-sided and a *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. *HSP70-2 expression in urothelial carcinoma cells and clinical specimens*

RT-PCR analysis revealed *HSP70-2* expression in all urothelial carcinoma cell lines tested (Fig. 1A). However, higher *HSP70-2* expression was found in HTB-1 and UMUC-3 as compared with HTB-9 and HTB-2 (densitometric analysis: Fig. 1A). *HSP-70-2* expression was not detected in normal human urothelial cells (Fig. 1A). The size of the PCR product was the same as in the testis (Fig. 1A). RT-PCR analysis further revealed that *HSP70-2* mRNA expression was detected in 70% of the patients with superficially invasive tumours, and 90% of the patients with muscle-invasive tumours not in ANCT as shown in representative tissue specimens in Fig. 1A. Statistical analysis revealed that a significant association was found between *HSP70-2* mRNA expression and tumour stages by Pearson χ^2-test ($P = .008$). *HSP70-2* expression was detected in 72% patients with low grade and 88% patients with high grade urothelial tumours. Interestingly, significant association was also found between *HSP70-2* mRNA expression and histological grades (low and high grades) by Pearson χ^2-test ($P = .028$). It is important to note that *HSP70-2* mRNA expression was significantly correlated with tumour stages and grades.

3.2. *HSP70-2 protein expression in human urothelial carcinoma cell lines and bladder cancer specimens*

All urothelial carcinoma cells revealed strong cytoplasmic HSP70-2 protein localisation in fixed and permeablised cells (Fig. 1B) whereas cell surface localisation of HSP70-2 was observed in live cells (Fig. 1C). Further, Western blot analysis revealed higher HSP70-2 protein expression in HTB-1 and

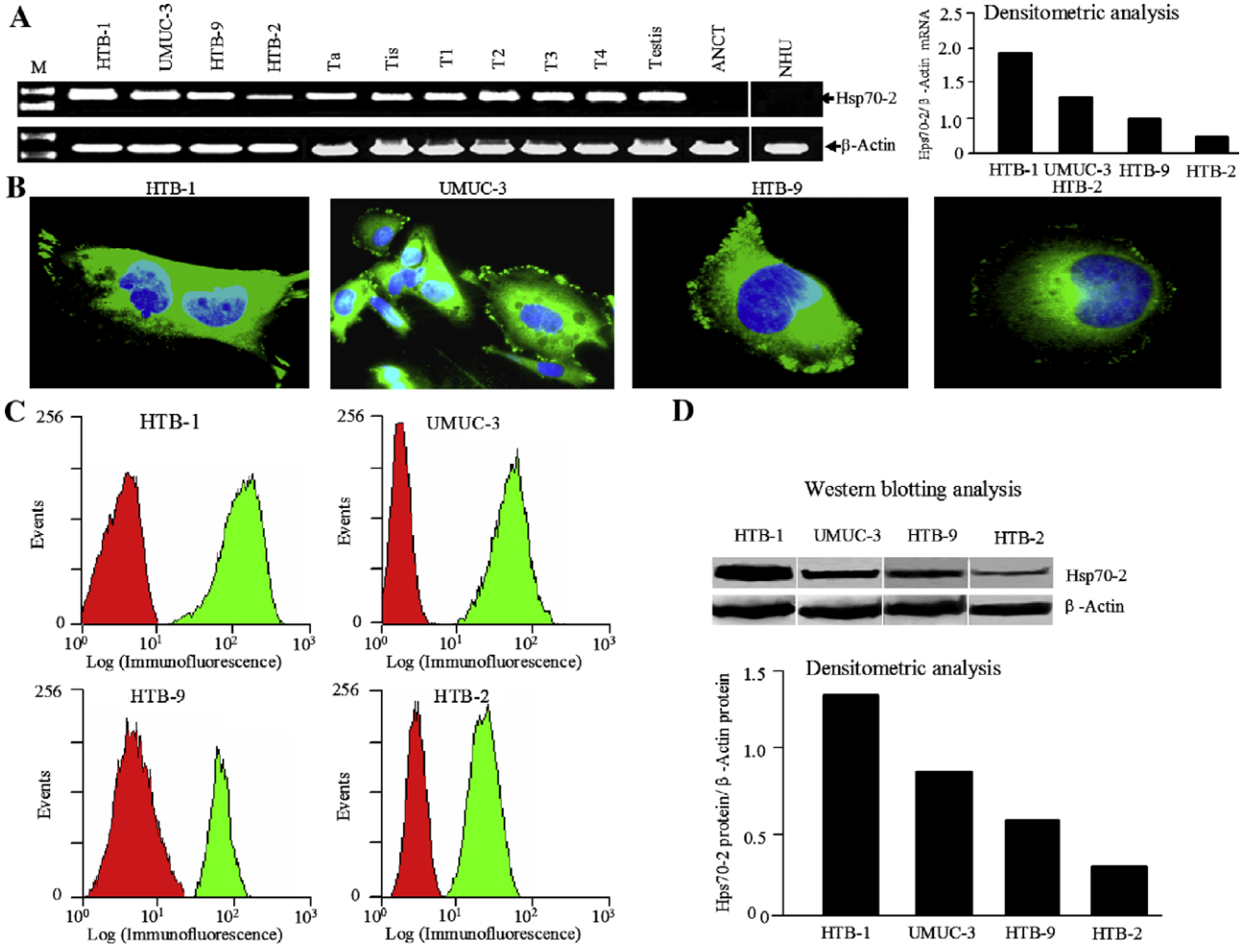

Fig. 1 – HSP70-2 mRNA and protein expression in urothelial carcinoma cell lines and carcinoma tissue. (A) *HSP70-2* was detected in all cell lines, in specimens of different stages, in testis but not in ANCT and normal human urothelial (NHU) cells. Histogram showing densitometric analysis of cell lines (B) HSP70-2 protein expression in fixed/permeablised urothelial carcinoma cells. (C) FACS analysis indicating surface localisation of HSP70-2 protein (green histogram); cells stained with secondary antibody only (red histogram). For all these experiments, the results of one representative experiment out of a minimum of three independent experiments are shown. (D) Western blotting and densitometric analysis of urothelial carcinoma cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

UMUC-3 cells as compared to HTB-2 and HTB-9 cells (densitometric analysis: Fig. 1D).

In bladder cancer specimens, HSP70-2 expression was observed in 70% of superficially invasive urothelial carcinoma and 90% of muscle-invasive urothelial carcinoma but not in ANCT as shown in Fig. 2A. No reactivity was observed in serial tissue sections incubated with control IgG and in ANCT specimen (Fig. 2A). In addition, HSP70-2 expression was detected in 72% patients with low grade and 88% patients with high grade urothelial carcinoma (Table 1).

We further determined the HSP70-2 immunostained cells by counting >500 cells from 5 random fields of each specimen under ×400 magnification in each tissue section. Immunohistochemistry analysis revealed that the overall HSP70-2 IRSs were significantly higher in muscle-invasive tumours (74.02 ± 1.37) as compared to superficially invasive tumours (61.98 ± 2.24) by Student's *t*-test (Table 1, $P < .0001$) (Fig. 2B). In addition, two groups were analysed based on HSP70-2 IRSs in urothelial carcinoma (group 1 moderate: less than 50% tumour cells expressing HSP70-2; and group 2 high: more than 50% tumour cells expressing HSP70-2). Our results showed that significantly higher number of urothelial carcinoma patients 87% (82/94) revealed higher HSP70-2 IRSs ($P < .0001$; 72.28 ± 1.08) as compared to less number of urothelial carcinoma patients 13% (12/94) having moderate HSP70-2 IRSs (43.75 ± 2.06) as shown in Fig. 2B using Student's *t*-test. Furthermore, HSP70-2 IRSs among the grades showed significant difference between low (60.58 ± 2.10) and high grade (75.43 ± 1.23) ($P < .0001$) by Student's *t*-test (Fig. 2B). Interestingly, significant association was also found between HSP70-2 IRS and low and high grade by Pearson χ^2-test ($P = .028$).

3.3. *Down-regulation of HSP70-2 decreases cell growth, colony formation, migration and invasion of urothelial carcinoma cells*

To determine the role of HSP70-2 in urothelial carcinoma cells, we used RNAi strategy to down-regulate the HSP70-2 expression. Four independent sets of HSP70-2-specific shRNAs were evaluated for their effectiveness in inhibiting HSP70-2 expression. In these experiments, treatment with HSP70-2shRNA3 and HSP70-2shRNA4 showed a greater impact as compared to HSP70-2shRNA1 and HSP70-2shRNA2 on ablation of HSP70-2 protein expression in Western blot analysis (Fig. 3A). These results indicate that HSP70-2shRNA3/HSP70-2shRNA4 effectively down-regulated HSP70-2 protein expression in HTB-1 as compared to control NCshRNA. However, none of the HSP70-2shRNA specific targets showed ablation of HSP70 and HSC70 proteins indicating that HSP70-2-specific shRNAs targets were specific to HSP70-2

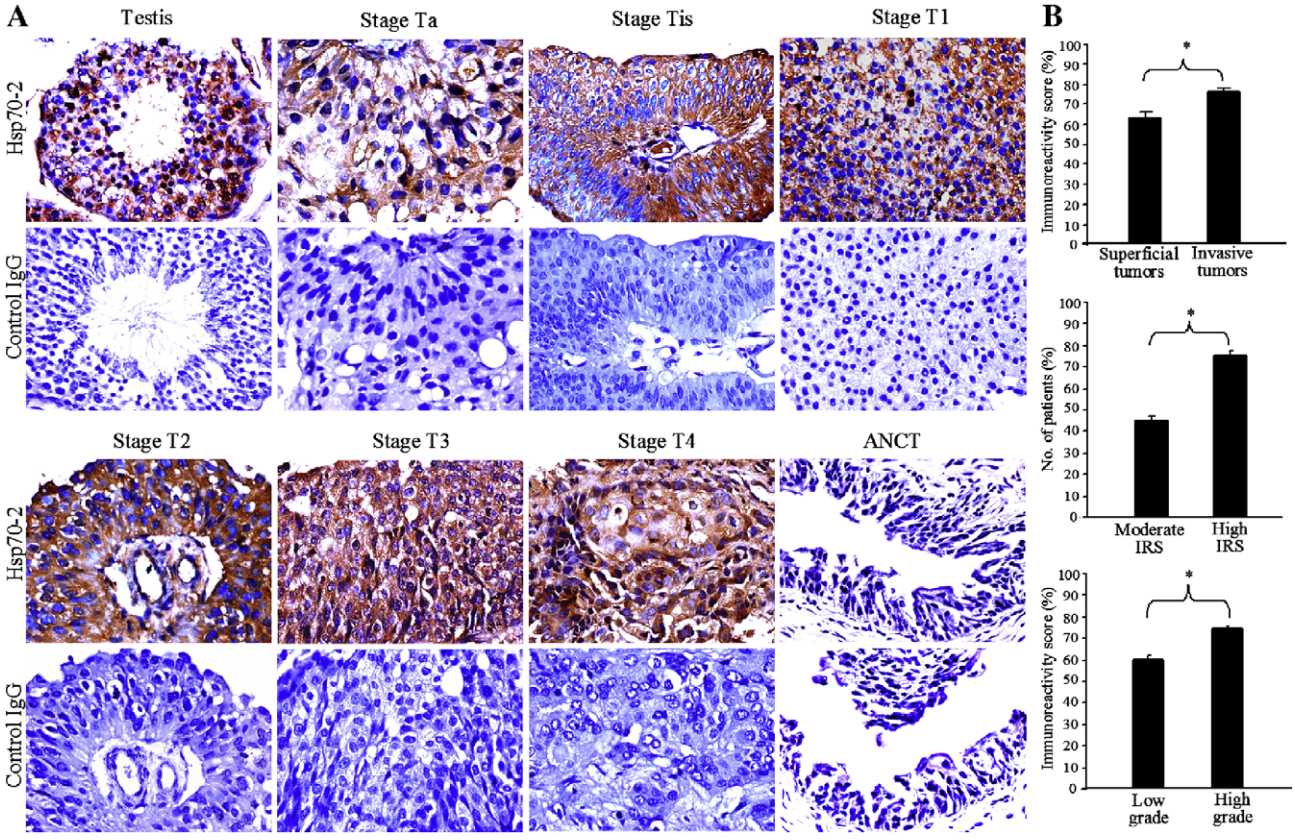

Fig. 2 – (A) Immunohistochemical detection of HSP70-2 protein in testis and bladder urothelial carcinoma tissue. Distinct cytoplasmic localisation of HSP70-2 protein in human testis and in different stages of bladder cancer was detected, no immunoreactivity in ANCT (original magnification, ×400; objective, 40×). Control IgG showed no immunoreactivity. (B) HSP70-2 IRSs and its association with pathologic stages and grades in superficial and invasive urothelial carcinoma. *Significant difference using Student's *t*-test ($P < .0001$).

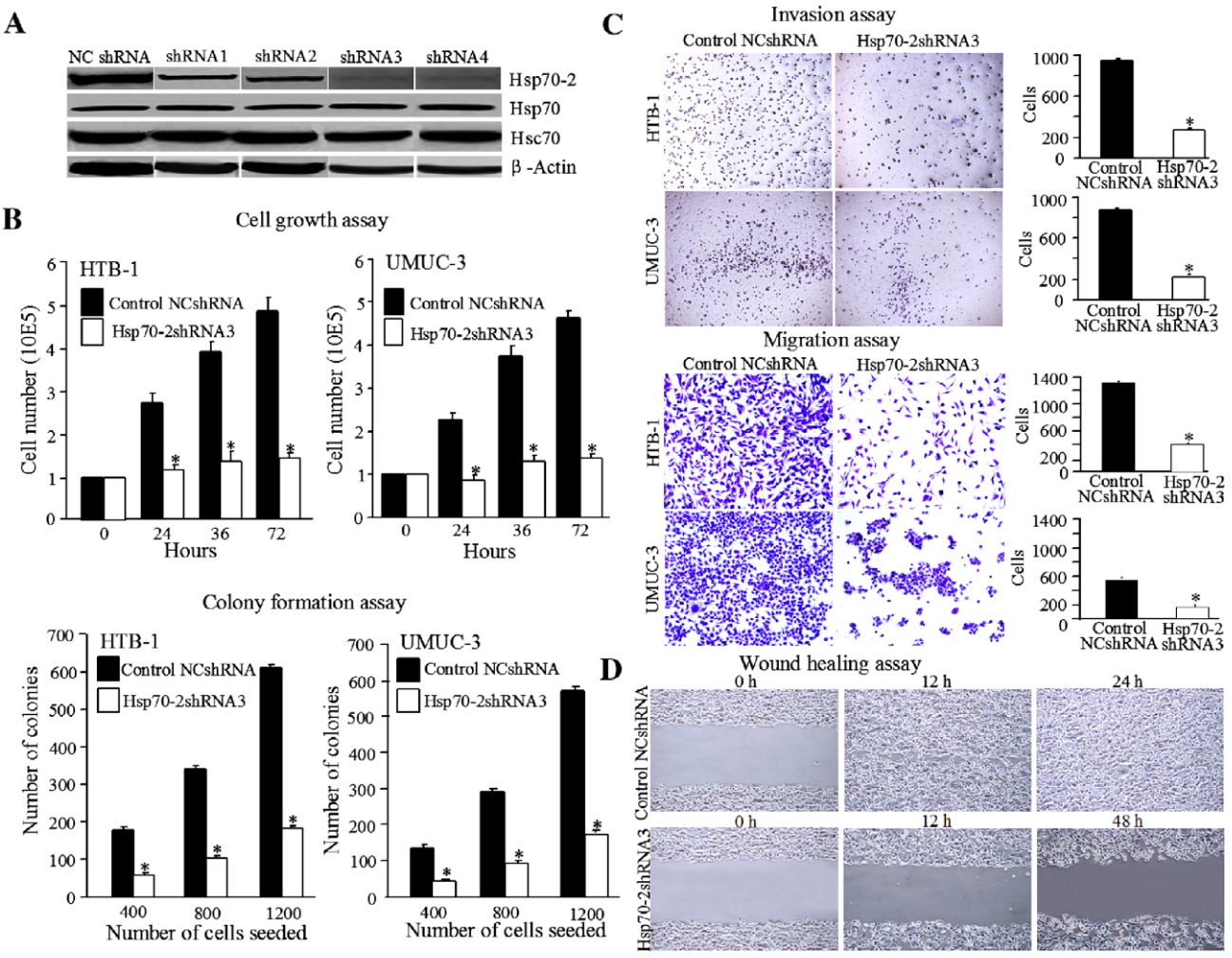

Fig. 3 – Depletion of HSP70-2 effects malignant properties of urothelial carcinoma cell. (A) Knockdown HSP70-2 protein in HSP70-2shRNA3 and HSP70-2shRNA4 treated cells. None of the HSP70-2shRNA resulted in reduction of HSP70 and HSC70 proteins. Control NCshRNA showed no effect on HSP70-2 expression. All subsequent experiments were carried out using HSP70-2shRNA3. (B) Quantitative determination analysis showed inhibition of cell growth and colony formation in HSP70-2shRNA3-treated cells. *n* = 3 independent experiments; each experiment was performed in triplicate. Points, mean; bars, SE.2 *Significant difference using Student's *t*-test (*P* < .0001). (C) Ablation of HSP70-2 protein in HTB-1 and UMUC3 cells reduced invasion and migration. (D) Wound healing assay shows that closing of scratch wound in HSP70-2shRNA3-transfected HTB-1 cells was not complete even after 48 h, whereas cells transfected with control NCshRNA successfully closed the scratch wound within 24 h. *Significant difference using Student's *t*-test (*P* < .0001).

testis specific proteins only (Fig. 3A). Hence, the subsequent experiments were restricted to HSP70-2shRNA3.

Subsequently, we examined the effect of HSP70-2shRNA3 on the growth of HTB-1 and UMUC-3 cells. Knockdown of HSP70-2 protein resulted in growth retardation of both HTB-1 and UMUC-3 cells. Cell growth was reduced to 29% and 30% for HTB-1 and UMUC-3 cells (Fig. 3B), respectively, post 72 h of treatment. Furthermore, no effect on the growth of normal human urothelial cells was observed employing HSP70-2shRNA3 (data not shown). Similarly, cellular colony formation was significantly suppressed by HSP70-2shRNA3 in both HTB-1 and UMUC-3 cells. The numbers of cell colonies of HSP70-2 knockdown cells were significantly reduced for various numbers seeded for HTB-1 (30–32% for 400–1000 cells) and UMUC-3 (30–32% for 400–1000 cells) (Fig. 3B).

The acquisition by cancer cells of an invasive phenotype is a critical step for tumour progression. The effects of HSP70-2 knockdown on migration and invasion abilities of invasive urothelial carcinoma cells were performed using HSP70-2shRNA3. Knockdown of HSP70-2 led to the inhibition of invading potential of HTB-1 and UMUC-3 cells (Fig. 3C) by 71% and 70%, respectively, and histogram shows that a significantly lower number of cells ($P < .0001$) migrated through inserts. Subsequently, transwell migration assay revealed 70% and 75% inhibition in motility of HSP70-2shRNA3 transfected HTB-1 and UMUC-3 cells, respectively (Fig. 3C), and histogram shows that a significantly lower number of cells ($P < .0001$) migrated through inserts. Subsequently, HSP70-2 knockdown also revealed decreased motility of HSP70-2shRNA3 transfected HTB-1 cells in wound healing assay. The wound was not

closed in HSP70-2shRNA3 transfected HTB-1 cells even after 48 h, whereas cells transfected with control NCshRNA successfully closed the wound within 24 h (Fig. 3D). Our findings revealed an essential role of HSP70-2 in the tumour cell motility and invasion, a key property of the aggressive cancer phenotype.

3.4. *Growth inhibition of HTB-1 urothelial carcinoma cell xenograft with HSP70-2 shRNA*

To further validate the biological significance of HSP70-2 in bladder carcinogenesis, we examined the effect of HSP70-2shRNA3 treatment on the *in vivo* growth of bladder tumour in xenograft-nude mouse model. Interestingly, mean tumour volumes and weight in HSP70-2shRNA3 treated mice were substantially reduced by day 49 ($P < .0001$) in comparison with those mice treated with control NCshRNA (Fig. 4A–C). Immunohistochemical analysis of HSP70-2 protein expression in tumour tissue revealed suppressed HSP70-2 protein expression and as compared to control NCshRNA groups (Fig. 4D). These results further confirm the significant role of HSP70-2 expression in bladder tumour growth and indicate that HSP70-2 may be a molecular target for effective treatment for urothelial carcinoma of bladder.

4. Discussion

Bladder cancer is currently one of the tumours with the most rapid increase in incidence. Majority of bladder cancers include superficial tumours which include the highly differentiated, non-invasive tumours to high-grade lamina propria invasive malignant lesions. The malignant tumours are associated with high metastatic potential and risk for invasive growth and are treated with adjuvant immunotherapy. Despite adjuvant therapy treatment, superficial tumours have recurrence rate of around 70% and one-third of them cause death due to tumour progression.[23] Since bladder cancer has high recurrence rate and requires lifelong treatment, bladder cancer is the most expensive cancer to treat on a per patient basis. Therefore, early

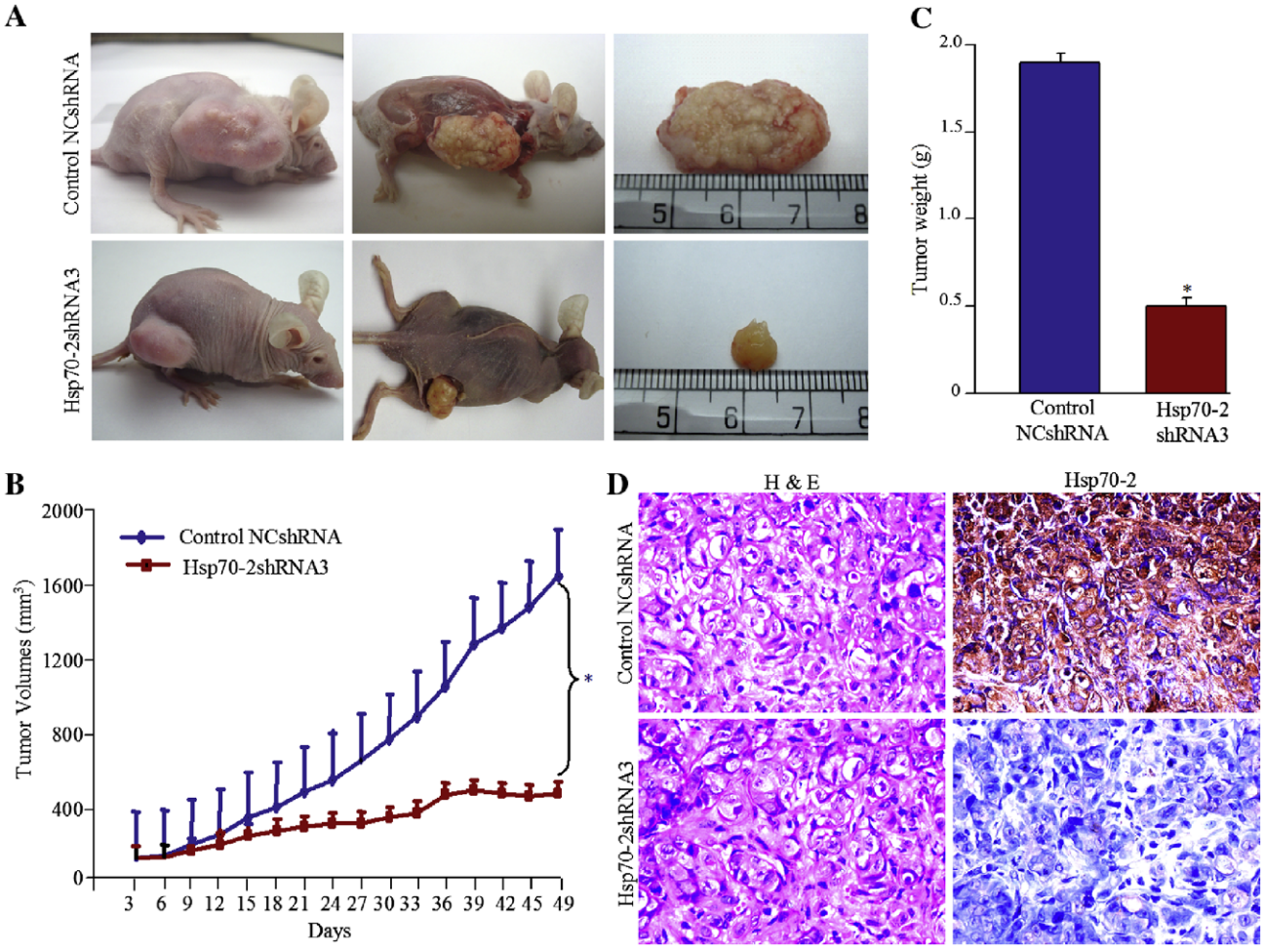

Fig. 4 – HSP70-2 knockdown inhibits human tumour growth in nude mice. (A) Representative photomicrograph showing reduction in tumour growth in mice injected intratumourally with HSP70-2shRNA3 as compare to control NCshRNA injected mice. (B and C) The tumour volume and weight was significantly greater at 49 d in mice injected with control NCshRNA compared with those injected with HSP70-2shRNA3. (D) Control NCshRNA-injected tumour revealed strong immunoreactivity against HSP70-2 protein, whereas no or very weak reactivity was observed in HSP70-2shRNA3-injected tumour. *Significant difference using Student's *t*-test ($P < .0001$).

detection and diagnosis that will allow initiating the appropriate therapy are essential for increasing the life expectancy of bladder cancer patients.[24]

Invasion of tumour cells resulting in metastasis involves differential gene expression that allows cells to bypass senescence, cell cycle arrest check points and cell signal pathways critical for controlling cell growth and differentiation.[25] In this context, identification of tumour proteins that are associated with early spread of cancer may prove to be powerful biomarkers to assess the progression of disease. In the present study, we evaluated HSP70-2 association and its role in bladder urothelial carcinoma. Our RT-PCR analysis revealed that HSP70-2 was expressed in both moderate to well-differentiated cancer cells and high-grade invasive urothelial carcinoma cells studied. Furthermore, HSP70-2 expression was also observed in clinical specimens of urothelial carcinoma patients with superficially invasive and muscle-invasive tumours. Our data suggest that HSP70-2 expression in urothelial carcinoma cells is involved in cell growth and cellular motility. This conclusion is based on meticulously controlled experiments employing siRNA-mediated knockdown of HSP70-2 expression in cancer cells, which significantly reduced the cell growth, colony formation and invasion of urothelial carcinoma cells. The expression of HSP70-2 in different clinical T stages and grades (Table 1) may support the role of HSP70-2 at cellular level in the regulation of growth and colony formation (Fig. 3B). The fact that knockdown of HSP70-2 expression significantly inhibited human HTB-1 xenograft tumour growth suggests that it may have vital function in bladder carcinogenesis (Fig. 4). Our findings further support that the HSP70-2 is not only expressed at the mRNA level but is also translated as evident from immunohistochemistry analysis of urothelial carcinoma of superficial and invasive origin (Fig. 2). The key finding of this study is that HSP70-2 expression is associated with stages and grades and is involved in cell growth and cellular motility, which may result in early spread of cancer.

Recently, gene-expression profiles obtained by DNA microarray analysis have provided information of various cancers, which may help to improve the cancer treatment strategies through the development of new drugs.[26] In addition, genome-wide expression analysis has resulted in the identification of a number of genes that function as oncogenes in the process of development and/or progression of bladder cancers.[27] However, the majority of the genes thus identified has not been investigated for their expression pattern, localisation and function in the tumour tissues. In a recent study, a member of HSP chaperone family, HSP70 was shown to be associated with various stages and grades in bladder cancer patients.[28] In addition, it has also been proposed that HSP70 protein promotes tumour cell growth by inhibiting programmed cell death and/or stabilising the lysosomal membranes.[29,30] However, a testis specific HSP70-2, another member of HSP chaperone family, neither its expression nor its function in urothelial carcinoma cells has been studied. In the present study, we investigated the association of HSP70-2 expression in bladder urothelial carcinoma. It is noteworthy that we observed HSP70-2 mRNA expression in 70% early stages and 90% late stages cancer patients. So far, only one study has reported HSP70-2 expression in small number of clinical specimens of primary [31% (5/16)] and metastatic [44% (4/9)] breast cancer tissue.[6] In contrast, our study demonstrated that majority of bladder urothelial carcinoma patients [80%] revealed HSP70-2 mRNA and protein expression irrespective of the superficial/invasive tumour stages and grades. This aspect is an important lead towards characterising tumour protein as a potential target for immunotherapy and development of cancer biomarker.

At present, no ideal tumour markers currently exist in clinical practise for early detection, monitoring or prognostic prediction of bladder urothelial carcinoma. Our demonstration of HSP70-2 protein expression in early stages of superficially invasive urothelial carcinoma is an important lead towards developing a tumour marker. Herein, we demonstrated a significant association of higher HSP70-2 protein expression (IRSs) with stages and grades (low and high grade). This is an important finding where, we demonstrate a testis specific HSP70-2 protein expression in bladder urothelial carcinoma patients with early stages and low grades of urothelial carcinoma, suggesting its possible implications in developing early diagnostics of bladder urothelial carcinoma malignancies.

The emergence of proteomic technologies allows the characterisation of altered protein expression and their aberrant localisation during the transformation of healthy cell into neoplastic cell, which may affect cellular function. In this regard, the cell surface compartment is of substantial interest in identification of tumour specific proteins for developing therapeutic targets for the cancer treatment. In this context, recent findings that the gene for the growth factor receptor (HER2) is expressed in breast tumours and HER2 protein is over expressed at the cell surface have resulted in an effective antibody-based therapy for breast cancer.[31] In addition, recent study in various cancer cell lines, reported surface localisation of HSP70-2 using comprehensive profiling of the cell surface proteome approach.[32] Interesting, our FACS data analyses also revealed that all urothelial carcinoma cells showed cell surface localisation of HSP70-2. Hence, our findings suggest that HSP70-2 expression may have important implications in developing antibody-based therapy or drug-based therapy for better cancer management programmes.

In conclusion, our findings define a possible role of HSP70-2 in association with the malignant properties of bladder urothelial carcinoma. These data open exciting possibilities for early detection and development of cancer therapies based on the targeting HSP70-2 expression.

Conflicts of interest statement

None declared.

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