Foster, CS; Dodson, AR; Ambroisine, L; Fisher, G; Müller, H; Clark, J; Attard, G; De-Bono, J; Scardino, P; Reuter, VE; Cooper, CS; Berney, DM; Cuzick, J (2009) Hsp-27 expression at diagnosis predicts poor clinical outcome in prostate cancer independent of ETS-gene rearrangement. British journal of cancer, 101 (7). pp. 1137-44. ISSN 0007-0920 DOI: 10.1038/sj.bjc.6605227

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Hsp-27 expression at diagnosis predicts poor clinical outcome in prostate cancer independent of ETS-gene rearrangement

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BACKGROUND: This study was performed to test the hypothesis that expression of small heat shock protein Hsp-27 is, at diagnosis, a reliable predictive biomarker of clinically aggressive prostate cancer.

METHODS: A panel of tissue microarrays constructed from a well-characterised cohort of 553 men with conservatively managed prostate cancer was stained immunohistochemically to detect Hsp-27 protein. Hsp-27 expression was compared with a series of pathological and clinical parameters, including outcome.

RESULTS: Hsp-27 staining was indicative of higher Gleason score (P<0.001). In tissue cores having a Gleason score > 7, the presence of Hsp-27 retained its power to independently predict poor clinical outcome (P<0.002). Higher levels of Hsp-27 staining were almost entirely restricted to cancers lacking ERG rearrangements (χ² trend = 31.4, P<0.001), although this distribution did not have prognostic significance.

INTERPRETATION: This study has confirmed that, in prostate cancers managed conservatively over a period of more than 15 years, expression of Hsp-27 is an accurate and independent predictive biomarker of aggressive disease with poor clinical outcome (P<0.001). These findings suggest that apoptotic and cell-migration pathways modulated by Hsp-27 may contain targets susceptible to the development of biologically appropriate chemotherapeutic agents that are likely to prove effective in treating aggressive prostate cancers.

British Journal of Cancer (2009) 101, 1137–1144. doi:10.1038/sj.bjc.6605227 www.bjcan.com
Published online 25 August 2009
© 2009 Cancer Research UK

Keywords: heat shock protein 27; prostate cancer; prognostic biomarker

Biologically and behaviourally, prostate cancer is a heterogeneous malignant disease (Foster et al, 2002). In the USA, in 2008, there were 186,320 new cases of prostate cancer and 28,660 deaths from this disease (Jemal et al, 2008). Equivalent figures from the United Kingdom reveal that 34,302 new cases were diagnosed in 2005 and that 10,000 men died of prostate cancer (http://info.cancerresearchuk.org/cancerstats/types/prostate/). Globally, prostate cancer is currently the fifth commonest malignancy and the most common in men, with more than 679,000 new cases estimated to have occurred in 2002 (Parkin et al, 2005). The clinical potential of an individual prostate cancer may range from relative indolence to highly aggressive, with progression occurring rapidly. Hence, there is an urgent requirement to develop reliable biomarkers that can accurately stratify prostate cancer at diagnosis and segregate men with aggressive cancers requiring urgent treatment from those who may be managed conservatively. Thus far, the only two parameters generally accepted to be valuable in the clinical management of prostate cancer are serum PSA and Gleason score following histopathological assessment of prostatic biopsy specimens. Although the extent of disease is more useful than clinical stage at diagnosis, and retains a low level of significance in multivariate analysis, neither of these are of clinical utility and are significantly inferior to Gleason score and PSA (Cuzick et al, 2006). Although both approaches have been clinically valuable, neither provides accurate predictive information with respect to individual prostate cancers.

Hsp-27 was originally discovered as an oestrogen-modulated protein in breast cancer (Adams et al, 1983), and thereafter identified to contribute to apoptosis (Tenniswood et al, 1992) and as a potential prognostic marker in prostate cancer cells (Morino et al, 1997). The functional relationship between Hsp-27, encoded by the gene HSPB1 located on human chromosome 7 at q11.23, and behavioural phenotype was suggested in the preliminary study that showed Hsp-27 expression to predict (P<0.0001) death from prostate cancer (Cornford et al, 2000). However, the cohort was heterogeneous and the sample size relatively small. In contrast, the current study has allowed a rigorous assessment of Hsp-27...
expression in a large cohort of well-characterised and conservatively managed patients (Cuzick et al, 2006) with respect to the prediction of tumour aggression. Our original observation that Hsp-27 predicts clinical recurrence in prostate cancer was supported by the finding from a subsequent study that the protein level increases after androgen ablation and is cytoprotective in hormone-refractory prostate cancer (Rocchi et al, 2004), and, more recently, by two studies on men undergoing radical prostatectomy (Glaessgen et al, 2008; Miyake et al, 2008).

The value of Hsp-27 expression as a predictive biomarker is not restricted to prostate cancer. In developing breast neoplasia, modulation of Hsp-27 in early proliferative lesions (O’Neill et al, 2003) occurs synchronously with that of ER-alpha to predict subsequent development of breast malignancy (P < 0.001). However, in established node-positive breast cancer, Hsp-27 does not predict survival (Tetu et al, 1995). In cervical neoplasia, association has been reported between over-expression of Hsp-27 and grade, although no survival data were included (Ono et al, 2009). With respect to treatment of malignant disease, increased expression of Hsp-27 has been linked to vincristine resistance in gastric cancer (Yang et al, 2009) and to 5-fluorouracil resistance in colon cancer (Tsuruta et al, 2008), and breast cancer cells initially over-expressing Hsp-27 became sensitive to doxorubicin after modulation of endogenous Hsp-27 levels by paclitaxel (Shi et al, 2008).

Prostate cancers often contain an ETS-gene fusion that involves the androgen-regulated promoter regions from the TMPRSS gene (chromosome 21q22-3) and joins a 9' ERF sequences (Tomlins et al, 2005). These translocations are common in established prostate cancer (Clark et al, 2007, 2008) and have been identified in PIN, but with a lower frequency (Cerveira et al, 2006; Perner et al, 2007; Mosquera et al, 2008). Taken together with evidence from transgenic mouse studies (Tomlins et al, 2008), these observations suggest that TMPRSS–ERG fusion alone may be insufficient for transformation from benign to malignant prostatic epithelium. However, the biological activity of alternatively spliced TMPRSS2– ERG fusion gene transcripts is pleiotropic (Wang et al, 2008), with the consequent effects on the emerging phenotypes of the involved neoplastic cells (initiation vs progression) depending on the corresponding rearrangement (Carver et al, 2009).

To test the hypothesis that Hsp-27 expression is a reliable predictive biomarker of clinically aggressive prostate cancer, we analysed an extensive set of tissue microarrays (TMAs) constructed from the archived diagnostic tissues obtained by the collaborating hospital trusts. This work was approved by the Clinical Research and Ethics Committee at the Royal Marsden Hospital and the Institute of Cancer Research.

**MATERIALS AND METHODS**

**Patient cohort**

To help identify and optimise markers that may be of use in the treatment of men with prostate cancer, we recently established a retrospective cohort of men who were managed only conservatively (Cuzick et al, 2006; Eastham et al, 2008). Improving on previous studies (Chodak et al, 1992; Albertsen et al, 1995, 2005; Adolfsson et al, 1997; Holmberg et al, 2002; Johansson et al, 2004; Bill-Axelson et al, 2005), our analyses included centrally assigned Gleason scores determined by modern grading criteria and allowed comparisons with several additional clinical parameters. In agreement with Johansson et al (2004) and Albertsen et al (2005), we found Gleason score to be an important determinant of cancer-specific mortality, but baseline PSA and, to a lesser extent, stage of disease added further predictive value. Tissue microarrays were constructed from an unselected transurethral resection of the prostate specimens taken from patients who had received no initial treatment in a cohort of conservatively managed men with prostate cancer. Men who had had hormone therapy before diagnostic biopsy were also excluded, because of the influence of hormone treatment in the interpretation of Gleason grade (Cuzick et al, 2006).

**Tissue micro-arrays for Hsp-27 analysis and for ETS-rearrangement FISH studies**

The original haematoxylin and eosin (H&E)-stained diagnostic tissues for all cases included in this study were reviewed by a panel of three urological pathologists (DMB, CSF and VER) as previously described to confirm each diagnosis of prostate cancer and to exclude all false positives (Berney et al, 2007b). Of the total number of cases reviewed, 133 (7%) were reassigned a non-malignant diagnosis and thereafter excluded. The remaining 1656 cases were further reviewed to confirm and standardise the Gleason score according to conventional criteria (Deshmukh and Foster, 1997). For those 1656 cases of cancer, there was a significant reassessment in the Gleason score across a wide spectrum, yielding a more accurate predictor of prognosis than the original scores in multivariate analysis (Berney et al, 2007a). Following mark-up of the tissue sections to identify the predominant patterns of prostate cancer and of non-malignant glandular epithelium, TMAs were constructed in 35 × 22 × 7 mm3 blocks of Lamb paraffin wax using a manual tissue microarrayer (Beecher Instruments, Sun Prairie, WI, USA). Up to four cores of 600 μm diameter were taken from each prostatic tissue. Reassign-ment of areas of cancer or non-malignant epithelium in each core was performed by histopathological examination of H&E- and p63/AMACR-stained sections that flanked the TMA slice used for FISH studies. The morphological criteria for selection of ‘normal’ and ‘malignant’ prostatic epithelium confirmed to previously published definitions (Foster, 2000; Foster et al, 2000, 2004). FISH studies to detect ERG and ETV1 gene re-arrangements were conducted as described previously (Attard et al, 2008a,b).

**Ethical approval**

Approval for the collection of the cohort was obtained from the Northern Multi-Research Ethics Committee, followed by approval from the local ethics committee at each of the collaborating hospital trusts. This work was approved by the Clinical Research and Ethics Committee at the Royal Marsden Hospital and The Institute of Cancer Research.

**Hsp-27 immunohistochemistry**

A mouse monoclonal antibody to Hsp-27 was purchased from Leica Microsystems (NCL-Hsp-27 – Leica Microsystems Newcastle Ltd, Newcastle Upon Tyne, UK). The ADVANCE Autostainer Universal Staining System, rabbit/mouse HRP was purchased from Dako (K4068, Dako UK Ltd, Ely, Cambridge, UK). High-temperature antigen retrieval was performed using a domestic stainless-steel pressure cooker at full pressure for 3 min in 10 mM EDTA solution (pH 7.0). The Hsp-27 antibody was diluted in antigen retrieval buffer (0.2% Tween-20 (TBS-T, pH 7.6) containing 0.12 mM NaCl and 0.05% Tween-20 (TBS-T)) and sections were incubated with the primary antibody for 40 min at room temperature, washed with TBS-T and
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incubated with ADVANCE link reagent for 20 min at room temperature, washed with TBS-T and incubated with ADVANCE enzyme reagent for 30 min at room temperature. Thereafter, sections were washed before applying a solution of 3,3′-diaminobenzidine tetrahydrochloride and H2O2 (DAB+, K3468, Dako UK Ltd) for 10 min to reveal sites of antibody binding. Following a rinse in deionised water, slides were removed from the Auto-stainer. Nuclei were counterstained with Mayer’s haematoxylin before mounting the slides in DPX. For the negative control used in each experiment, the primary antibody was replaced with Antibody Diluent. All sections were scored using CSF and ARD.

Analysis of Hsp-27 expression Specimens were considered positive only if at least 5% of the epithelial cells (either normal or malignant) unequivocally expressed Hsp-27 staining. The 5% cut-off was chosen because it conforms to the International European Organization for Research and Treatment of Cancer-Gynaecological Cancer Cooperative Group recommendations (van Diest et al, 1997). Furthermore, this cut-off was used as the criterion to distinguish positive and negative immunohistochemical staining in our previous studies of prostate cancer (Cornford et al, 2000), thus ensuring consistency of criteria between studies. For each tissue section, staining was assessed as negative, weakly positive or only focally positive (low-level expression), or strongly positive (high-level expression), and scored as 0, 1, 2 or 3, respectively. For positive cores, the cellular distribution of Hsp staining in benign or malignant prostatic epithelium was assessed.

Statistical analyses The primary end points for this study were time to death from prostate cancer and time to death from any cause. Univariate and multivariate analyses were performed by proportional hazard (Cox) regression analysis (Cox and Oakes, 1984). All follow-up times commenced at the point of 6 months proportional hazard (Cox) regression analysis (Cox and Oakes, 1984), cause. Univariate and multivariate analyses were performed by time to death from prostate cancer and time to death from any cause. For positive cores, the cellular distribution of Hsp staining in benign or malignant prostatic epithelium was assessed.

RESULTS

Hsp-27 expression in prostate tissue

Analysis of the cores of tissues contained in the 24 blocks comprising the arrays showed that 743 patients were represented on the arrays that included 1251 cancer cores, 18 PIN, 624 non-PIN hyperplasia and 792 cores with morphologically benign hyperplasia (Cuzick et al, 2006). The distribution of cytoplasmic Hsp-27 expression within the various morphological groups ranging from benign to malignant is presented in Figure 1. In this analysis, Hsp-27 expression was identified in 64% of the cores containing morphologically unremarkable prostatic epithelia, and in 50% of the cores of hyperplastic tissues (Figure 2). Of the 18 PIN cores only 7 (39%) were positive, while 200 (16%) of the prostate cancer cores were positive. When the data for the malignant tissues were analysed with respect to individual cases, 553 patients remained in the analysis, with 441 (79.7%) of the cases being negative for Hsp-27 and 112 (20.3%) of the cases showing cytoplasmic positivity. The patients’ demographics and tumour characteristics, together with details of their Hsp-27 expression, are presented in Table 1. Hsp-27 expression was associated with Gleason score (P = 0.001), whereas no association was found with age, baseline PSA, clinical stage or extent of disease.

Overall effect of Hsp-27 Absence of Hsp-27 expression was associated with a better survival from prostate cancer (Figure 3) and overall survival. In the univariate analyses, Hsp-27 cytoplasmic positivity was a significant prognostic factor for poor survival from prostate cancer (HR = 1.89, 95% CI = 1.32–2.70, P < 0.001) and overall survival (HR = 1.60, 95% CI = 1.26–2.05, P < 0.001). In multivariate analyses that included Gleason score, extent of disease, baseline PSA and age at diagnosis, Hsp-27 cytoplasmic positivity remained an independent prognostic factor for poor cancer-specific survival (ΔH₁(1df) = 4.49, P = 0.03) and overall survival (ΔH₂(1df) = 7.45, P = 0.005).

Subgroup analyses Analysis of Hsp-27 expression according to Gleason score: The independent prognostic significance of Hsp-27 cytoplasmic positivity for prostate cancer survival in a multivariate model was maintained in prostate cancers with Gleason score > 7 (ΔH₁(1df) = 7.08, P = 0.03 for prostate cancer survival), but not in prostate cancers with Gleason score 7 (ΔH₂(1df) = 0.43, P = 0.50) or in prostate cancers with Gleason score < 7 (ΔH₃(1df) = 0.11, P = 0.75), as shown schematically in Figure 4.

Analysis of Hsp-27 expression according to ETS-re-arrangement status: Both Hsp-27 expression and ETS (ERG and ETVI) gene status were available for 435 of the 553 patients (Table 2). When the cancers were stratified according to ETS-gene status, in univariate analyses the presence of Hsp-27 cytoplasmic positivity failed to reach significance for prostate cancer survival in both non-rearranged ETS (ΔH₁(1df) = 2.35, P = 0.12) and rearranged ETS (ΔH₂(1df) = 0.68, P = 0.40) groups. However, interplay of the data (Table 2) revealed a significant difference in the distribution of Hsp-27 staining in the two groups (χ² = 18.3, P < 0.001). With increasing expression of Hsp-27 there was a progressive decline in ETS-gene rearrangement frequency (χ²(trend) = 31.4, P < 0.001), such that most of the strongly staining cancers were found in ERG non-rearranged tumours.

DISCUSSION

This study has shown that, at diagnosis, Hsp-27 expression is a powerful independent predictive biomarker for survival from prostate cancer (P < 0.001) and for overall survival (P < 0.001). Using a large cohort of men gathered from across the United Kingdom over a period of more than 15 years, Hsp-27 expression was used to accurately segregate men with poor prognosis of prostate cancer and who required active treatment from those with relatively indolent disease that could be managed conservatively, with confidence. In this study, Hsp-27 expression correlated strongly with Gleason grade (P = 0.001), but not with baseline PSA value (P = 0.50), des-PSA being an independent variable in the original cohort of patients. The latter observation reflects the complex relationships between Hsp-27 and androgen receptor in the transactivation of PSA (Zoubeidi et al, 2007). The current findings also reveal that while most morphologically benign tissues strongly express Hsp-27, lower proportions of hyperplasia and PIN exhibit staining (Figure 1), suggesting that expression of the protein may be down-regulated in the in-situ neoplastic epithelial cells, which are the precursors of invasive prostate cancer (Foster et al, 2002).

There is strong evidence that Hsp-27 is an important gatekeeper of cancer cell migration and survival through differential phosphorylation and by its relative level of expression. Evidence from different cell systems indicates that Hsp-27 controls cell
locomotion by a common mechanism. In fibroblasts (Hirano et al, 2004), endothelium, neutrophils and epithelial cells, phosphorylation and polymerisation of Hsp-27 modulates transformation of G → F actin (Tak et al, 2007) to generate the mechanical force required for cell motility to occur through organisation of cytoskeletal components (Doshi et al, 2009). In endothelial cells, Hsp-27 co-localises with and regulates assembly of actin filaments following phosphorylation by p38 MAP kinase (Guay et al, 1997; Landry and Huot, 1999). Thereafter, the phosphorylated and non-phosphorylated forms of Hsp-27 become differentially distributed throughout the lamellipodia, which, in the presence of fascin (Vignjevic et al, 2006), enable cell migration (Nagy et al, 2008).

Non-phosphorylated Hsp-27, excluded from the leading edge of the lamellipodia, maintains short-branched actin molecules, whereas phosphorylated Hsp-27 at the base of the lamellipodia stabilises actin networks composed of long unbranched filaments (Pichon et al, 2004). When Hsp-27 phosphorylation is inhibited and functional interaction with actin impaired, migration dependent on F-actin polymerisation is prevented (Chen et al, 2009).

Enhanced levels of Hsp-27, together with its relocation from the cytoplasm to the nucleus (Wissing and Jaattela, 1996), is associated with increased resistance to apoptosis (Samali and Cotter, 1996). However, inhibition of apoptosis by Hsp-27 involves at least two parallel pathways. In the first, phosphorylated dimers of Hsp-27 interact with Daxx and block Fas-mediated apoptosis, but with no effect on Fas-induced FADD and caspase-dependent apoptosis (Charette et al, 2000). In the second, following interaction of...
phosphorylated Hsp-27 with the cytochrome c/Apaf-1/dATP complex, the Fas-adaptor FADD down-regulates the procaspase 9-dependent apoptotic pathway (Gibbons et al, 2000; Jolly and Morimoto, 2000; Charette et al, 2001). In the prostate, down-regulation of apoptosis is associated with elevated levels of Hsp-27, such that PC-3 and LNCaP cells become resistant to both chemical- and radiation-induced apoptosis (Gibbons et al, 2000; Jolly and Morimoto, 2000).

In non-malignant cells Hsp-27 is phosphorylated by MAP kinase p38 (Rouse et al, 1994; Huot et al, 1995), thus maintaining cellular homeostasis and integrity. Thereafter, Hsp-27 exists in an auto-phosphorylating signal complex together with Akt, p38 MAPK and MK2, in which Akt becomes phosphorylated on Ser473 by MK2 (Morimoto, 2000). When Hsp-27 dissociates from the complex before Akt activation, apoptosis is induced. In androgen-independent prostate cancer, lethal progression is associated with reduced apoptosis (Berges et al, 1995) and enhanced tumour cell proliferation (McLoughlin et al, 1993; Berney et al, 2009). Mechanistically, Hsp-27 activity is regulated by post-translational phosphorylation at serine residues Ser15, Ser78 and Ser82 and at theonine residue Thr143 (Landry et al, 1992). However, this process appears to be promiscuous in cancer cells, being dependent on the particular kinase activation pathway invoked and the individual cell system involved. In response to extra- or intra-cellular information, signal transducer Stat 3, controlled by its inhibitor MK2, in which Akt becomes phosphorylated on Ser473 by MK2 (Wu et al, 2007). When Hsp-27 dissociates from the complex before Akt activation, apoptosis is induced. In androgen-independent prostate cancer, lethal progression is associated with reduced apoptosis (Berges et al, 1995) and enhanced tumour cell proliferation (McLoughlin et al, 1993; Berney et al, 2009).

Comparison of the distribution and intensity of Hsp-27 expression with ETS status (Clark et al, 2007, 2008; Attard et al, 2008a, b) has confirmed a statistically significant (P<0.001) inverse relationship between these two parameters. As the numbers of cancers containing Ets rearrangements were similar to the numbers with Ets non-rearranged in the Hsp-27-negative group, but declined rapidly with increasing expression of Hsp-27 (Table 2), it is possible that strong expression of Hsp-27 might prevent subsequent rearrangement of Ets genes if these occur subsequent to changes in Hsp-27. With respect to behavioural phenotype, strong expression of Hsp-27 and ETS-gene rearrangement are each independently associated with aggressive and rapidly lethal prostate cancer.

Not only is Hsp-27 a powerful biomarker of aggressive prostate cancer, but it is also a potential target for novel therapeutic intervention. Studies involving antisense oligonucleotides or siRNA gene knockdown showed that Hsp-27 expression promotes androgen-independent progression of prostate cancer (Rocchi et al, 2004, 2005). Inhibition of HSPB1 expression modulated apoptosis and abrogated the malignant phenotype of human prostate cancer cells, thus identifying Hsp-27 as a potential therapeutic target. Separately, the biphenyl isoxasole KRIIB3 inhibits tumour cell migration by blocking protein kinase C-dependent phosphorylation of Hsp27 (Shin et al, 2005) to induce mitotic arrest and to enhance apoptosis (Shin et al, 2008). Recently, it has been shown that pyrrolo-pyrimidines, a novel class of p38 MAPK/MAPK-activated protein kinase 2 (MK2) DIAS3, alters genomic expression of Hsp-27 and facilitates phosphorylation at Ser78 (Song et al, 2004).

Table 1 Relationship of Hsp-27 level with demographics and tumour characteristics

| Variable                  | Total (n = 553) | Negative (n = 441) | Positive (n = 112) | P-value |
|---------------------------|-----------------|--------------------|--------------------|---------|
| Mean age ± s.d. (years)   | 69 ± 5          | 69 ± 5             | 70 ± 5             | 0.43    |
| Mean follow-up ± s.d.     | 95 ± 49         | 99 ± 48            | 82 ± 47            | <0.001  |
| Early hormone management  |                 |                    |                    | 0.21    |
| Yes                       | 119             | 90 (76%)           | 29 (24%)           |         |
| No                        | 434             | 351 (81%)          | 83 (19%)           |         |
| Gleason score             |                 |                    |                    | 0.001   |
| <7                        | 244             | 212 (87%)          | 32 (13%)           |         |
| =7                        | 158             | 120 (76%)          | 38 (24%)           |         |
| >7                        | 151             | 109 (72%)          | 42 (28%)           |         |
| Clinical stage            |                 |                    |                    | 0.78    |
| T1                        | 144             | 110 (76%)          | 34 (24%)           |         |
| T2                        | 134             | 107 (80%)          | 27 (20%)           |         |
| T3                        | 69              | 52 (77%)           | 15 (23%)           |         |
| Unknown                   | 206             | 171 (83%)          | 35 (17%)           |         |
| Baseline PSA (ng ml⁻¹)    |                 |                    |                    | 0.22    |
| ≤4                        | 173             | 147 (85%)          | 26 (15%)           |         |
| >4 – 10                   | 102             | 76 (75%)           | 26 (25%)           |         |
| >10 – 25                  | 120             | 97 (81%)           | 23 (19%)           |         |
| >25 – 50                  | 95              | 73 (77%)           | 22 (23%)           |         |
| >50 – 100                 | 63              | 48 (76%)           | 15 (24%)           |         |
| Cancer in biopsy (%)      |                 |                    |                    | 0.13    |
| ≤5                        | 123             | 104 (85%)          | 19 (15%)           |         |
| >5 – 20                   | 139             | 110 (79%)          | 29 (21%)           |         |
| >20 – 40                  | 79              | 68 (86%)           | 11 (14%)           |         |
| >40 – 75                  | 83              | 62 (75%)           | 21 (25%)           |         |
| >75 – 100                 | 122             | 91 (75%)           | 31 (25%)           |         |
| Unknown                   | 7               | 6 (86%)            | 1 (14%)            |         |

Abbreviation: PSA = prostate-specific antigen. *Relationship with Hsp-27 calculated for patients with available clinical stage. #Relationship with Hsp-27 calculated for patients with available percentage of cancer in biopsy.

Figure 3 (A) Prostate cancer survival and (B) overall survival according to Hsp-27 expression.
and candidate chemotherapeutic agent, inhibits cell migration by blocking activation of this pathway (Xu and Bergan, 2006), emphasising the validity of this proposed therapeutic approach.

This study analysing the largest cohort yet reported of conservatively managed patients with prostate cancer for more than 15 years has shown the accuracy of Hsp-27 expression as a prognostic biomarker of aggressive disease at initial diagnosis. Further, the analysis indicates two divergent phenotypes of lethal prostate cancer involving either ETS rearrangement or high Hsp-27 expression. These findings are of biological interest not only for studying the initiation and progression of prostate cancer but also for clinically identifying men who require immediate aggressive management to control potentially lethal disease. As this is a relatively small subset (~25–35%) of men diagnosed with prostate cancer, use of this biomarker provides the biological rationale to focus the available resources on actively treating this high-risk group, while allowing the majority of prostate cancer patients (~65–75%) to be managed conservatively.

ACKNOWLEDGEMENTS

This work was funded by Cancer Research UK, National Cancer Research Institute, a specialised program of Research Excellence grant from the US National Cancer Institute, Grand Charity of Freemasons, Rosetrees Trust, The Bob Champion Cancer Trust, The Orchid Appeal, David Koch Foundation and North West Cancer Research Fund. Funding bodies had no involvement in the design and conduct of the study; in collection management, analysis and interpretation of the data; or in preparation, review and approval of the paper. We thank Jill Gosney for her assistance in preparing this paper.

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Statistical analysis $\chi^2$ (trend) = 31.4, P < 0.001; $\chi^2$ (positive vs negative) = 18.3, P < 0.001.
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