Cytoplasmic accumulation of Sequestosome 1 (p62) is a predictor of biochemical recurrence, rapid tumor cell proliferation and genomic instability in prostate cancer

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Running title: Sequestosome 1 in prostate cancer

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Conflict of interest statement: The authors do not declare any conflicts of interest.
Statement of translational relevance

There is an urgent need for predictive progression markers to discriminate between aggressive and non-aggressive prostate cancers. By immunohistochemistry on a tissue microarray containing 12,427 prostate cancers, the authors demonstrate that strong cytoplasmic p62 staining was tightly linked to high Gleason grade, advanced pathological tumor stage, positive nodal status, positive resection margin, and early PSA recurrence. Analysis of cytoplasmic accumulation of p62 is a strong predictor of an adverse prognostic behavior of prostate cancer.
Abstract

Purpose: Sequestosome 1 (p62) is a multifunctional adapter protein accumulating in autophagy-defective cells.

Experimental Design: To evaluate the clinical impact and relationship with key genomic alterations in prostate cancer, p62 protein levels were analyzed by immunohistochemistry on a tissue microarray containing 12,427 prostate cancers. Data on ERG status and deletions of PTEN, 3p13, 5q21 and 6q15 were available from earlier studies.

Results: p62 immunostaining was absent in benign prostatic glands but present in 73% of 7,822 interpretable prostate cancers. Strong cytoplasmic p62 staining was tightly linked to high Gleason grade, advanced pathological tumor stage, positive nodal status, positive resection margin, and early PSA recurrence (p<0.0001 each). Increased levels of p62 were significantly linked to TMPRSS2-ERG fusions, both by FISH and immunohistochemical analysis (p<0.0001 each). For example, moderate or strong p62 immunostaining was seen in 28.5% of cancers with TMPRSS2:ERG fusion detected by FISH and in 23.1% of cancers without such rearrangements (p<0.0001). Strong p62 staining was significantly linked to presence of all tested deletions, including PTEN (p<0.0001), 6q15 (p<0.0001), 5q21 (p=0.0002), and 3p13 (p=0.0088), 6q15 (p<0.0001), suggesting a link between p62 accumulation and loss of genomic stability. The prognostic role of p62 protein accumulation was striking and independent of Gleason grade, pT stage, pN stage, surgical margin status and preoperative PSA, irrespective of whether preoperative or postoperative parameters were used for modeling.

Conclusions: Our study identifies cytoplasmic accumulation of p62 as a strong predictor of an adverse prognostic behavior of prostate cancer independently from established clinico-pathological findings.
Introduction

Prostate cancer is the most prevalent cancer in men in Western societies (1). Although the majority of prostate cancers behave in an indolent manner, a small subset is highly aggressive and requires extensive treatment (2, 3). Established prognostic parameters are limited to Gleason grade and tumor extent on biopsies, preoperative prostate-specific antigen (PSA), and clinical stage. Because these data are statistically powerful, but often insufficient for optimal individual treatment decisions, it is hoped that a better understanding of disease biology will eventually lead to the identification of clinically applicable molecular markers that enable a more reliable prediction of prostate cancer aggressiveness.

Sequestosome 1 (SQSTM1 or p62) is a multifunctional adapter protein. Although the full spectrum of its functions is not known, there is growing evidence that p62 regulates apoptosis and cell survival through catabolic metabolism of molecules involved in NF-kB, mTOR, MAPK, and possibly also other signaling pathways (4-7). p62 localizes to the membranes of autophagosomes, and is itself cleared by autophagy (6, 7). Accordingly, accumulation of p62 protein is believed to result from impaired autophagy, a condition that has been described in many tumor types, including cancers of the lung (8), breast (9, 10), colon (11), mouth (12) and liver (13). In line with an oncogenic role, p62 accumulation has been linked to poor prognosis in some of these tumor types (8, 10, 12).

Little is known about the role and clinical significance of p62 accumulation in prostate cancer. Two previous studies involving a total of 152 malignant and 28 benign prostate specimens reported discrepant results. One study on 73 patients described a gradual increase of the staining intensity from normal to hyperplastic, and malignant prostate tissue (14). The other study – on 107 patients – could not confirm these data (15). To clarify the clinical relevance of p62 expression in prostate cancer, we took advantage of our preexisting tissue microarray containing >12,000 prostate cancer specimens connected to a database with extensive clinical follow up and molecular data. Our findings demonstrate that high levels of p62 protein expression are strongly linked to an adverse phenotype and early PSA recurrence of prostate cancer, and suggest that these associations might be driven by a strong link of p62 protein accumulation to chromosomal instability.
Materials and Methods

Patients. Radical prostatectomy specimens were available from 12,427 patients, undergoing surgery between 1992 and 2012 at the Department of Urology and the Martini Clinics at the University Medical Center Hamburg-Eppendorf. Follow-up data were available for a total of 12,344 patients with a median follow-up of 36 months (range: 1 to 241 months; Supplementary Table 1). Prostate specific antigen (PSA) values were measured following surgery and PSA recurrence was defined as a postoperative PSA of 0.2 ng/ml and increasing at first of appearance. All prostate specimens were analyzed according to a standard procedure, including a complete embedding of the entire prostate for histological analysis (16). The TMA manufacturing process was described earlier in detail (17). In short, one 0.6 mm core and 5μ thick, was taken from a representative tissue block from each patient. The tissues were distributed among 27 TMA blocks, each containing 144 to 522 tumor samples. For internal controls, each TMA block also contained various control tissues, including normal prostate tissue. The molecular database attached to this TMA contained results on ERG expression in 10,678 (18), ERG break apart FISH analysis in 7,099 (expanded from (19)) and deletion status of CHD1 (5q21) in 7,932 (expanded from (20)) MAP3K7 (6q15) in 6,069 (expanded from (21)), PTEN (10q23) in 6,704 (expanded from (22)) and FOXP1 (3p13) in 7,081 (expanded from (23)) cancers, and Ki67 labeling Index (Ki67 LI) data in 4,426 (expanded from (24)) cancers.

Immunohistochemistry. Freshly cut TMA sections were immunostained on one day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C; 2 bar, in pH 9 Tris-EDTA-Citrate buffer. Primary antibody specific for SQSTM1 / p62 (mouse monoclonal antibody, Abcam, Cambridge, UK; cat#56416; dilution 1:4050 in Dako REAL™ Antidiluent S2022) was applied at 37°C for 60 minutes. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer’s directions (19). P62 stained the tumor cell cytoplasm in all (100%) cells of a tissue spot. Staining intensity of all cases was thus semiquantitatively assessed in four categories: negative, weak, moderate and strong. The percentage of positive tumor cells (typically 100%) was not separately recorded.
Statistics. Statistical calculations were performed with JMP 9® software (SAS Institute Inc., NC, USA). Contingency tables and the chi²-test were performed to search for associations between molecular parameters and tumor phenotype. Survival curves were calculated according to Kaplan-Meier. The Log-Rank test was applied to detect significant differences between groups. Analysis of variance (ANOVA) test was applied to search for associations between cell proliferation and p62 staining. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular and clinical variables. Separate analyses were performed using different sets of parameters available either before or after prostatectomy.

Results

Technical issues. A total of 7,822 (62.9%) of tumor samples were interpretable in our TMA analysis. Reason for non-informative cases (4,605 spots; 37.1%) included lack of tissue samples (2,411 spots; 19.4%) or absence of unequivocal cancer tissue in the TMA spot (757 spots; 6.1%) or insufficient staining of tissue samples (1,437 spots; 11.6%).

P62 expression in prostate cancer. P62 was expressed in the cytoplasm of normal prostate luminal cells with mostly weak intensity (range no staining to moderate staining). No expression was found in normal basal or stroma cells. Cytoplasmic p62 immunostaining was seen in 5,716 of our 7,822 (73%) interpretable prostate cancers and was considered weak in 51.4%, moderate in 19.6% and strong in 2.1% of cancers. Representative images of p62 immunostainings are shown in Figure 1.

Association with TMPRSS2:ERG fusion status and ERG protein expression. To evaluate whether p62 staining is associated with ERG status in prostate cancers, we compared the p62 results with data from previous studies (expanded from (18, 19)). Data on TMPRSS2:ERG fusion status obtained by FISH were available from 7,099 patients and by immunohistochemistry from 10,678 patients. Data on both ERG FISH and IHC were available from 6,778 cancers, and an identical result (ERG IHC positive and break by FISH) was found in 6,463 of 6,778 (95.4%) cancers. Strong p62 staining was significantly linked to TMPRSS2:ERG rearrangement and ERG expression in prostate cancers (p<0.0001 each; Figure 2), but the difference was
only minimal in absolute numbers and may first of all be due to the high number of analyzed cancers rather then to relevant biological differences. For example, moderate or strong p62 immunostaining was seen in 28.5% of cancers with TMPRSS2:ERG fusion detected by FISH and in 23.1% of cancers without such rearrangements (p<0.0001).

**Associations with tumor phenotype.** Strong p62 staining was significantly linked to high Gleason grade, advanced pathological tumor stage, positive nodal status, elevated preoperative PSA-level and positive resection margin status (p<0.0001, each; Table 1). In the subgroup of ERG negative cancers, advanced tumor stage, high Gleason grade, positive lymph node status, positive resection margin status and a high preoperative PSA-Level were strongly associated with p62 accumulation (Supplementary Table 2). Also in ERG positive cancers, pathological tumor stage, high Gleason grade, positive lymph node status (p<0.0001 each) and preoperative PSA-Level (p=0.0039) remained to be significantly linked to p62 staining (Supplementary Table 3), while differences in the resection margin status were not statistically significant.

**Associations with other key genomic alterations of prostate cancer.** Earlier studies had provided evidence for distinct molecular subgroups of prostate cancers defined by TMPRSS2:ERG fusions and several genomic deletions. Others and us had previously described a strong link between PTEN and 3p13 deletions and ERG positivity as well as between 5q21 and 6q15 deletions and ERG negativity (21-23). To study, whether p62 expression might be particularly linked to one of these genomic deletions, p62 data were compared to preexisting findings on PTEN (10q23), FOXP1 (3p13), MAP3K7 (6q15) and CHD1 (5q21) deletions. In the analysis of all tumors, deletions of all four genes were significantly linked to high p62 expression (p<0.009 for each gene; Figure 3a). Most of these associations were retained in the subsets of ERG negative and ERG positive cancers, although the differences failed to reach statistical significance for 3p13 in ERG-negative cancers and for 6q15 in ERG-positive cancers (Figure 3b and 3c).

**Association with tumor cell proliferation.** Data on Ki67 immunohistochemistry of the cancers included in our TMA were available from a previous study (24). Data on both Ki67 labeling index (Ki67LI) and p62 staining were available from 4,874 cancers. High levels of p62 staining were significantly linked to increased tumor cell proliferation (p<0.0001). This association was independend from the Gleason grade
(p≤0.0009) and was also retained in separate analyses of ERG-negative and ERG-positive cancers (Supplementary Table 4).

**Associations with PSA recurrence.** Follow-up data were available for 7,044 patients with interpretable p62 immunostaining on the TMA. There was a statistical significant association between high p62 expression and early PSA recurrence if all tumors were analyzed (p<0.0001; Figure 4a), but also in the subgroups of ERG negative (p<0.0001) and ERG positive (p<0.0001) cancers (Figure 4b and 4c). Because of the strong association between p62 overexpression and PTEN deletions, and the important prognostic impact of PTEN deletion in prostate cancer, we performed additional analyses to estimate the combined impact of alterations of p62 and PTEN. For this analyses, we grouped tumors with moderate to strong staining (p62 high) and tumors with negative or weak staining (p62 low) and combined these groups with the PTEN status (non deleted / deleted) into four subsets of cancers: 1) cancers with low p62 expression lacking PTEN deletion, 2) cancers with high p62 expression lacking PTEN deletion, 3) cancers with low p62 expression and PTEN deletion and 4) cancers with high p62 expression and PTEN deletions. The prognostic differences of these groups were then calculated separately in subsets of ERG-negative (Figure 4d) and ERG-positive cancers (Figure 4e). It showed that cancers with high p62 expression and concomitant PTEN deletion had a significantly worse prognosis than those cancers harboring only one of these alterations. This held true in the subset of ERG-negative cancers (p=0.0267) as well as in the subset of ERG-positive cancers (p<0.0001).

**Multivariate analysis.** Four multivariate analyses were performed evaluating the clinical relevance of p62 expression in different scenarios (Table 2). No 1 was utilizing all postoperatively available parameters including pathological tumor stage, pathological lymph node status (pN), surgical margin status, preoperative PSA value and pathological Gleason grade obtained after the morphological evaluation of the entire resected prostate. Scenario 2 was utilizing all postoperatively available parameters with exception of nodal status. The rational for this approach was that the indication and extent of lymph node dissection is not standardized in the surgical therapy of prostate cancer and that excluding pN in multivariate analysis can markedly increase case numbers. Two additional scenarios had to purpose to model the preoperative situation. Scenario 3 included p62 expression, preoperative PSA, clinical tumor stage (cT stage) and Gleason grade obtained on the prostatectomy specimen. Since postoperative determination of a tumors Gleason grade is “better”
than the preoperatively determined Gleason grade (subjected to sampling errors and consequently under-grading in more than one third of cases (25)), another multivariate analysis was added. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, cT stage and p62 expression. If all tumors were analyzed, all scenarios suggest a strong evidence of p62 to represent an independent predictor of prognosis (Table 2). Separate analysis of ERG positive and ERG negative cancers revealed, that p62 expression has independent prognostic relevance in both ERG negative and ERG positive cancers in all scenarios (Table 2).

Discussion

The results of this study demonstrate that cytoplasmic accumulation of p62 protein is a predictor of unfavorable tumor phenotype and early PSA recurrence in prostate cancer, which is independent of established clinico-pathological features.

Our immunohistochemical analysis revealed cytoplasmic p62 staining in 73% of our 7,822 analyzable prostate cancers. This frequency is somewhat lower than what has been observed in two earlier IHC studies reporting positive p62 staining in 91% of 45 prostate cancers employing conventional large section analysis (14), and in 100% of 107 prostate cancers analyzed in a tissue microarray format (15). These variances may be first of all attributable to differences in the antibodies, immunostaining protocols, and scoring criteria. That comparable high fractions of p62 positive cancers can be found with both large section and TMA approaches demonstrates that our analysis provided representative data not markedly influenced by sampling error issues that can potentially occur in studies evaluating small tissue cores measuring only 0.6 mm in diameter per patient.

The lack of unequivocal staining in normal prostate is in line with the study by Kitamura et al. (14). These authors also failed to find any cytoplasmic positivity in 9 normal prostate glands, however, reported some faint nuclear staining which was also observed in cancer cells. Since a nuclear function of p62 is not known, it seems possible that such staining may be non-specific. The finding that cytoplasmic p62 was clearly up-regulated in cancers as compared to normal tissues, and that increasing levels of p62 paralleled cancer aggressiveness, i.e. high Gleason score, advanced stage, metastatic phenotype, and increased tumor cell proliferation, is
consistent with a relevant role of p62 protein for prostate cancer development and progression.

Considering the difference of more than 30 percent points in the recurrence free survival between p62 negative and strongly positive cancers 60 month after surgery, the prognostic impact of p62 staining is comparable to the strongest established prognostic markers in prostate cancer such as PTEN deletions and p53 alterations (16, 22, 26, 27). The high relevance of p62 protein is further emphasized by strong data from our multivariate analysis. Multivariate analyses of data derived from prostatectomy samples raise several issues, however. Firstly, it is obvious, that the inclusion of all strong prognostic features that only become available after surgery such as pT, pN, surgical margin or the validated Gleason grade (based on the thorough analysis of the entire prostate) makes it difficult for any biomarker to be established as an independent predictor of prognosis. The inclusion of the pN category also limits the power of analysis. Including pN can substantially reduce the number of study cases because lymph node dissection is not a routine procedure in prostate cancer surgery. Postoperative analyses utilize many parameters that are unavailable at the moment when therapy decisions are made. Competing parameters for a clinically useful prognostic biomarker would thus rather include parameters that are available before surgery, such as the Gleason grade obtained from the core needle biopsy, the preoperative PSA value and the clinical T category. In the optimal case, potential prognostic biomarkers should even be evaluated on preoperative needle biopsies. From a practical point of view, this is hardly feasible, however, because diagnostic needle biopsy samples are usually distributed among many different pathology laboratories. Even if these samples could be collected for the purpose of a study, these precious core needle biopsies would be exhausted after only few analyses. In our cohort, multiple models are applied for multivariate analyses in order to compensate as much as possible for inherent limitations. That a strong and independent association between p62 overexpression and early PSA recurrence in prostate cancers was found in all analyzed scenarios, including various combinations of preoperatively and postoperatively available parameters, strongly supports our notion of p62 expression representing a clinically relevant biomarker in prostate cancer. Considering that a clinical biomarker must be analyzed on biopsy material and before treatment decisions are taken, it is of note, that our approach of analyzing molecular features on one minute TMA tissue specimen measuring 0.6 mm in diameter closely models the molecular analyses of core needle biopsies where comparable amounts of tissues are evaluated. As our TMA samples were not exactly
taken from the “worst” area of each tumor but randomly from within a representative cancer area, our TMA spot might be as representative as possible of the “worst” area of a clinical cancer identified in a set of cancer biopsies.

A relevant role of p62 in cancer biology is further suggested by an increasing number of reports assigning a relevant prognostic or biologic role to p62 in various different cancer types such as lung, breast, colon, oral cavity, and colon cancer (8-13). The exact mechanism of how p62 affects prostate cancer cells is not clear. It has been demonstrated, however, that p62 affects apoptosis and cell survival through degradation of molecules involved in various signaling pathways and that such alterations can induce oncogenic signaling (4-7). For example, Lee et al. showed that p62 is an early response gene involved in cell survival (28), and Inami et al. found in mice that persistent activation of the anti-apoptotic Nrf2 stress response results from p62 accumulation (13). Moreover, several studies linked p62 overexpression to activation of mTOR and NF-kB signaling, two pathways that are associated to cancer development (5, 29, 30). Cell line studies have even suggested that elevated p62 expression was associated with invasive growth properties (13, 31).

In this study, we have not executed own functional experiments, but the large number of prostate cancers included in our project together with extensive molecular information on our tumors enabled us to obtain functional information “in silico”. This approach of “functional molecular epidemiology” first demonstrated, that p62 expression - and probably also function - is completely independent of ERG activation. More than half of prostate cancers, particularly in young patients, carry gene fusions linking the androgen-regulated TMPRSS2 gene with the transcription factor ERG (18, 32). This genomic rearrangements result in an androgen-driven overexpression of ERG in affected cells (33). ERG activation leads to a substantial reprogramming of prostate epithelial cells with altered expression of a multitude of genes and many prognostic factors have a substantially different impact in ERG positive and negative cancers (34-37). Almost identical expression levels and identical prognostic impact of p62 protein in ERG positive and negative cancers argues against a relevant impact of ERG driven cell reprogramming on p62 gene function. The minimally higher rate (5.5%) of p62 positive cases amongst ERG positive cancers is likely due to a small fraction of poorly immune-reactive tissues that are inevitably included in IHC studies and cause a significant positive association between antibodies in case of very large studies.
Our “in silico” data further demonstrate, that cytoplasmatic p62 accumulation is strongly linked to classical parameters of genomic instability – such as prevalence of chromosomal deletions - and to elevated cell proliferation. Deletions of certain small and large chromosomal regions are a hallmark of prostate cancer. Data from next generation sequencing studies demonstrate that such deletions are more prevalent than mutations of coding genes and many of these deletions have been linked to either ERG positive (i.e. PTEN and 3p13) or ERG negative cancers (i.e. 6q15 and 5q23). That high p62 expression is linked to a higher prevalence of all analyzed deletions is consistent with an impact of p62 expression on mechanisms regulating genomic integrity. Mathew et al. found that autophagy-deficient cells accumulate p62, damaged organelles and radical oxygen species (ROS), which jointly contribute to DNA damage (29). In addition, Belaid et al. demonstrated that defective autophagy results in co-accumulation of p62 and its ubiquitination target protein RHOA (38), which controls the formation, position, and contraction of the actomyosin ring during cytokinesis (39). The authors reported that increased levels of RHOA caused cytokinesis failure resulting in daughter cells with multiple nuclei and an increased frequency of chromosomal gains and losses in nearly all chromosomes (38).

That cancers harboring both high (moderate to strong) p62 expression and PTEN deletions had a particularly worse prognosis suggests a functional interaction between PTEN loss and p62 overexpression. This notion is in line with previous work showing that p62 directly regulates the mTORC1 complex (40), which is also controlled by PTEN. It can, thus, be assumed that loss of PTEN and concomitant p62 overexpression results in a particularly strong activation of mTORC1 and its downstream target genes, including the MYC oncogene.

That p62 accumulation was linked to a massive increase of the proliferation rate, as determined by Ki67 immunohistochemistry, independently from the Gleason grade further supports a relevant role of p62 for regulation of pathways involved in growth and proliferation control. In line with this assumption, accumulation of p62 in autophagy-defective mouse models of hepatocellular carcinoma and lung cancer has been shown to activate non-canonical NF-kB signaling and compensatory cell proliferation in order to escape apoptosis (29, 41).

In summary, our study provides evidence that p62 is an independent major prognosticator in prostate cancer. We thus propose, that p62 expression analysis has the potential for clinical routine application - either alone, or more likely, in
combination with other biomarkers. Our large-scale tissue microarray approach will continue to proof highly instrumental for continuously identifying optimal prognostic biomarkers. Large scale molecular databases associated to large TMAs also enable limited “in silico” functional analyses.

Acknowledgments

We thank, Julia Schumann, Sünje Seekamp, and Inge Brandt for excellent technical assistance.
References


### Table 1. Clinico-pathological association of p62 immunostaining in prostate cancers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n evaluable</th>
<th>p62 (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>negative</td>
<td>weak</td>
</tr>
<tr>
<td><strong>All cancers</strong></td>
<td>7,822</td>
<td>26.9</td>
<td>51.4</td>
</tr>
<tr>
<td><strong>Tumor stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>4,977</td>
<td>29.9</td>
<td>53.5</td>
</tr>
<tr>
<td>pT3a</td>
<td>1,804</td>
<td>23.9</td>
<td>48.7</td>
</tr>
<tr>
<td>pT3b</td>
<td>964</td>
<td>16.4</td>
<td>46.0</td>
</tr>
<tr>
<td>pT4</td>
<td>47</td>
<td>31.9</td>
<td>42.6</td>
</tr>
<tr>
<td><strong>Gleason grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3+3</td>
<td>1,824</td>
<td>40.2</td>
<td>49.2</td>
</tr>
<tr>
<td>3+4</td>
<td>4,379</td>
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<td>55.4</td>
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<tr>
<td>4+3</td>
<td>1,222</td>
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</tr>
<tr>
<td>≥4+4</td>
<td>360</td>
<td>13.6</td>
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<td><strong>Lymph node metastasis</strong></td>
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<td>4,460</td>
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<td>N+</td>
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<tr>
<td><strong>Preop. PSA level (ng/ml)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>&lt;4</td>
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<tr>
<td>4-10</td>
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<tr>
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<tr>
<td>&gt;20</td>
<td>570</td>
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<td><strong>Surgical margin</strong></td>
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<tr>
<td>negative</td>
<td>6,194</td>
<td>27.3</td>
<td>52.0</td>
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<tr>
<td>positive</td>
<td>1,480</td>
<td>25.3</td>
<td>49.2</td>
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Table 2. Multivariate Cox regression analysis including established prognostic parameters and the p62 status in all prostate cancers, the ERG negative- and ERG positive subset. Scenario 1 includes all postoperatively available parameters (pathological tumor (pT) stage, lymph node (pN) stage, surgical margin (R) status, preoperative PSA value and Gleason grade obtained after the morphological evaluation of the entire resected prostate. Scenario 2 excluded the nodal status from analysis. Scenario 3 included preoperative PSA, clinical tumor (cT) stage and Gleason grade obtained on the prostatectomy specimen. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, and cT stage.

<table>
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<tr>
<th>Tumor subset</th>
<th>Scenario</th>
<th>n analyzable</th>
<th>p -value</th>
<th>pT Stage</th>
<th>cT Stage</th>
<th>Gleason grade prostatectomy</th>
<th>Gleason grade biopsy</th>
<th>pN Stage</th>
<th>R Status</th>
<th>p62-Expression</th>
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<td>4,363</td>
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<td>-</td>
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<td>-</td>
<td>&lt;0.0001</td>
<td>0.002</td>
<td>0.0004</td>
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<td>2</td>
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<td>-</td>
<td>&lt;0.0001</td>
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<td></td>
<td>3</td>
<td>6,869</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
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<tr>
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<td>4</td>
<td>6,775</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>ERG-negative</td>
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<td>2,178</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>0.004</td>
<td>0.68</td>
<td>0.01</td>
</tr>
<tr>
<td>cancers</td>
<td>2</td>
<td>3,426</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
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<td>0.0005</td>
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<td>3</td>
<td>3,394</td>
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<td>&lt;0.0001</td>
<td>-</td>
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<td>-</td>
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<tr>
<td></td>
<td>4</td>
<td>3,350</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
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Figure legends

**Figure 1.** Representative images of p62 immunostaining: a) Negative staining in prostate cancer, b) weak staining in non-tumorous prostate epithelium, c) moderate staining in prostate cancer, d) strong positive staining in prostate cancer.

**Figure 2.** Association between p62 expression levels and ERG-fusion state. Comparison of p62 expression levels in ERG-negative and ERG-positive prostate cancers. ERG-fusion state was determined either by immunohistochemistry, or by FISH for ERG gene breakage.

**Figure 3.** Association between p62 immunostaining and PTEN, 5q21, 6q15, and 3p13, in a) all prostate cancers, b) ERG negative cancers, and c) in ERG positive cancers.

**Figure 4.** Biochemical (PSA) recurrence free survival stratified for p62 expression in a) all cancers (n=7,144) as compared to subsets of b) ERG-fusion negative (n=3,308), c) ERG-fusion positive prostate cancers (n=2,645). In order to estimate the impact of co-alterations of p62 and ERG, cancers were grouped according to the PTEN deletion status and the p62 expression status (p62 low, i.e., negative or weak staining; p62 high, i.e., moderate or strong staining), and the prognostic impact was compared in subsets of d) ERG-fusion negative prostate cancer (n=2,080) and e) ERG-fusion positive prostate cancers (n=3,913).
Fig. 2

![Bar chart showing distribution of ERG expression levels in different groups.](chart.png)

- **ERG-negative** group (n=3863)
  - ERG-IHC: strong (30%), moderate (25%), weak (45%)
  - ERG-FISH: strong (25%), moderate (25%), weak (50%)
  - p<0.0001

- **ERG-positive** group (n=3134)
  - ERG-IHC: strong (40%), moderate (30%), weak (30%)
  - ERG-FISH: strong (30%), moderate (30%), weak (40%)
  - p<0.0001

- **Normal** group (n=2472)
  - ERG-IHC: strong (20%), moderate (40%), weak (40%)
  - ERG-FISH: strong (20%), moderate (40%), weak (40%)
  - p<0.0001

- **BA** group (n=2137)
  - ERG-IHC: strong (10%), moderate (50%), weak (40%)
  - ERG-FISH: strong (10%), moderate (50%), weak (40%)
  - p<0.0001
Fig. 4

(A) PSA recurrence-free survival for different PTEN deletion levels: negative (n=1114), weak (n=1574), moderate (n=572), and strong (n=48). The p-value is <0.0001.

(B) PSA recurrence-free survival for different PTEN deletion levels: negative (n=551), weak (n=1448), moderate (n=582), and strong (n=64). The p-value is <0.0001.

(C) PSA recurrence-free survival for different p62 expression levels: high PTEN deletion (n=95), high PTEN normal (n=399), low PTEN deletion (n=169), and low PTEN normal (n=1417). The p-value is 0.0267.

(D) PSA recurrence-free survival for different p62 expression levels: high PTEN deletion (n=310), high PTEN normal (n=713), low PTEN deletion (n=486), and low PTEN normal (n=2404). The p-value is <0.0001.
Cytoplasmic accumulation of Sequestosome 1 (p62) is a predictor of biochemical recurrence, rapid tumor cell proliferation and genomic instability in prostate cancer


Clin Cancer Res  Published OnlineFirst April 29, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-0620

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