High sulfite oxidase expression could predict postoperative biochemical recurrence in patients with prostate cancer

Hirofumi Kurose[1, 2], Yoshiki Naito[1, 3], Jun Akiba[3], Reiichiro Kondo[1], Sachiko Ogasawara[1], Hironori Kusano[1], Sakiko Sanada[1], Hideyuki Abe[3], Tatsuyuki Kakuma[4], Kosuke Ueda[2], Tsukasa Igawa[2], Hirohisa Yano[1]

[1] Department of Pathology, Kurume University School of Medicine, Kurume, Japan

[2] Department of Urology, Kurume University School of Medicine, Kurume, Japan

[3] Department of Diagnostic Pathology, Kurume University Hospital, Kurume, Japan

[4] Biostatistics Center, Kurume University School of Medicine, Kurume, Japan

Corresponding Author: Yoshiki Naito

Department of Pathology, Kurume University School of Medicine

67 Asahi-machi, Kurume 830-0011, Japan

Tel: +81-942-31-7546

Fax: +81-942-32-0905

E-mail: kurose_hirofumi@med.kurume-u.ac.jp

Running title: SUOX expression in prostate cancer

Conflicts of Interest and Source of Funding: All authors have declared that they have no conflicts of interest.

Abstract

Sulfite oxidase (SUOX) is a metalloenzyme that plays a role in ATP synthesis via oxidative phosphorylation in mitochondria and has been reported to also be involved in the invasion and differentiation capacities of tumor cells. Here, we performed a clinicopathological investigation of SUOX expression in prostate cancer and discussed the usefulness of SUOX expression as a predictor of biochemical recurrence following surgical treatment in prostate cancer. This study was conducted using Tissue Micro Array specimens obtained from 97 patients who underwent radical prostatectomy at our hospital between 2007 and 2011. SUOX staining was used to evaluate cytoplasmic SUOX expression. In the high-expression group, early biochemical recurrence was significantly more frequent than in the low-expression group ($p = 0.0008$). In multivariate analysis, high SUOX expression was found to serve as an independent prognostic factor of biochemical recurrence (hazard ratio = 2.33, 95% confidence interval = 1.32–4.15, $p = 0.0037$). In addition, Ki-67 labeling indices were significantly higher in the high-expression group than in the low-expression group ($p = 0.0058$). Therefore, SUOX expression may be a powerful prognostic biomarker for decision-making in postoperative follow-up after total prostatectomy and with regard to the need for relief treatment.

Key word: Prostate cancer, SUOX, Biomarker, Biochemical recurrence, Oxidative phosphorylation

Introduction

Prostate cancer is the most common solid cancer and the second most frequent cause of death of men in the United States of America. In Japan, the widespread use of prostate-specific antigen (PSA) testing led to prostate cancer becoming the most prevalent cancer among men in 2016 [1]. Most patients have organ-confined or locally advanced tumors at diagnosis, and prostate cancer is generally considered to progress relatively slowly. Therefore, when surgery is selected for the treatment of localized prostate cancer, the prognosis is generally good [2]. However, the rates of postoperative biochemical recurrence are 16–31% and 25–53% after 5 and 10 years, respectively [3 4]. Some of these cases develop into castration-resistant prostate cancer after clinical recurrence, often leading to poor outcomes. Thus, biochemical recurrence is often used to justify the application of salvage therapies, such as endocrine therapy and radiotherapy.

Although recurrence of prostate cancer after radical treatment is generally assessed based on increase in PSA (biochemical recurrence) [5-7], studies have identified a variety of predictive factors for biochemical recurrence. Significant factors include a designation of "poor risk" in the D'Amico classification, positive surgical margins (RM1), pre-operative PSA score, Gleason score at prostatectomy, and pathological staging. Of these, positive surgical margins (RM1) are the most important predictive factor for biochemical recurrence (recurrence rate: 1.5–6.0×) [8-16]. However, there are few reports of effective biomarkers that can be used to predict biochemical recurrence.

Sulfite oxidase (SUOX), a metalloenzyme found in mitochondria, interacts with molybdenum and heme as a coenzyme[17 18] . SUOX is a cytochrome *b*5 enzyme that belongs to the oxotransferase superfamily, which includes dimethyl sulfoxide reductase, xanthine oxidase, and nitrite reductase. SUOX converts electrons generated as a result of oxidation of sulfurous acid into sulfuric acid via cytochrome c and facilitates the transport of electrons used in ATP synthesis via oxidative phosphorylation; therefore, SUOX has been identified as one of several indicator molecules with respect to oxidative

phosphorylation in ATP synthesis[19-21]. Although numerous research papers concerning SUOX deficiency have been published to date, SUOX deficiency has also been described in reports as a factor involved in the invasion and differentiation capacity of cancer cells [22]. In tongue cancer in particular, SUOX has been shown to be related to oncogenesis based on the results of comprehensive analysis of mRNA extracted from healthy and dysplastic mucous membranes as well as sites of cancerous invasion. However, few studies have examined SUOX expression in cancer cases, and SUOX expression profiles in tumor cells have not been elucidated [23].

Accordingly, in this study, we evaluated SUOX expression in patients with biologically localized prostate cancer who had undergone surgical resection and investigated the feasibility of using serum PSA value, in conjunction with Gleason score and various histopathological factors, as a predictive factor of biochemical recurrence.

Materials and Methods

Patients and tissue samples

Ninety-seven patients who underwent prostatectomy at Kurume University Hospital (Kurume, Japan) between January 2007 and December 2011 were enrolled in this study. As part of this study, the pathological diagnoses of the patients were re-examined. The following patients were excluded from this study beforehand: 1) patients who had undergone hormonal therapy and/or radiotherapy before surgery, 2) patients found to be at stage pT0 during surgery, and 3) patients from whom specimens could not be created using tissue microarray (TMA). All patients were pathologically diagnosed with prostatic adenocarcinoma. Paraffin-embedded samples of primary prostate cancer tissue from 97 patients were used to construct a TMA containing 22 cores per slide (two primary tissue cores per patient). Histopathological evaluations were performed by three pathologists (H.K., Y.N. and R.K.). Pathological diagnosis was performed according to the 2016 World Health Organization Classification of Tumors of the Urinary System and Male

Genital Organs [24].

This study was approved by the Research Ethics Committee of Kurume University (#18161), which conforms to the guidelines of the Declaration of Helsinki.

Immunohistochemical analysis

Paraffin-embedded tissue samples were cut to a thickness of 4 μm, examined on coated slide glass, and labeled with anti-SUOX antibodies (1:600; Abcam, Cambridge, MA, USA) and anti-Ki-67 antibodies (NCL-Ki67-MM1; dilution 1:200; Leica Biosystems, Nussloch, Germany) using a BenchMark ULTRA (Ventana Automated Systems, Inc., Tucson, AZ, USA). Briefly, the slides were heat treated using Ventana's ULTRA cell conditioning 1 retrieval solution (CC1; Ventana Automated Systems, Inc.) for 36 min at 95°C and incubated with anti-SUOX antibodies for 32 min at 37°C. An automated system with a Ventana UltraVIEW 3,3′ -diaminobenzidine (DAB) detection kit was used, including horseradish peroxidase-multimer as the secondary antibody and DAB as the chromogen. Slides were incubated with secondary antibody for 30 min at 37°C. For quantification of staining with anti-SUOX antibodies, the intensity score (scale 0–2: score0; no expression, score1; low expression, score2; high expression) and population score (scale 0–5: score0; 0, score1; 0~1/100, score2; 1/100~1/10, score3; 1/10~1/3, score4; 1/3~2/3, score5; 2/3~) were added to generate a Histology Score (H-score) with H = intensity + population score (0–7). All immunohistochemical analyses were

evaluated by two experienced pathologists who were unaware of the patients' clinical conditions. The scores of the two pathologists were added, and each patient was given a total expression score consisting of 15 levels from 0 to 14. We considered only cytoplasmic expression of SUOX as positive. Ki-67 labeling index (LI) was calculated as the percentage of tumor cells that showed positive expression. Further, double staining using anti SUOX and Ki-67 antibodies was performed in this study to assess the correlation between SUOX expression and Ki-67 LI. Resected prostate cancer tissue samples were labeled with Ki-67 and SUOX antibodies using the BenchMark ULTRA and Bond-III autostainer for double-staining analysis. BenchMark ULTRA was used for Ki-67. Briefly, the slide was heat-treated using Ventana's CC1 retrieval solution for 64 min, and incubated with Ki-67 antibody for 30 min. This automated system used the streptavidin-biotin complex method with DAB. Immunostaining with SUOX was performed on the same fully automated Bond-III system, and incubated with the SUOX antibody for 30 min. The automated system used a Bond Polymer Refine Red Detection Kit with Fast Red as the substrate chromogen. Counterstaining was performed with hematoxylin. Finally, nuclear Ki-67 was labeled brown with DAB, and cytoplasmic SUOX was labeled red with Fast Red.

Statistical analysis

We examined the correlations between SUOX expression and clinicopathological characteristics, such as age at diagnosis, serum PSA level at diagnosis, Gleason score at radical prostatectomy, pathological T stage, lymphatic invasion, peripheral nerve invasion, positive surgical margins, Ki-67 LI, and biochemical recurrence using χ^2 tests or Fisher exact tests. The cut-off value of SUOX was determined by receiver operating characteristic curve analysis. Cancer survival analysis was performed using the Kaplan-Meier method, log-rank test, and Cox's proportional hazards model. The threshold for statistical significance was set at $p < 0.05$. Biochemical recurrence was defined as an increase in PSA level over 0.2 ng/mL after two different measurements at least 3 months apart. The statistical software used was JMP Pro 13 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

The clinicopathological characteristics of the 97 patients are summarized in **Table 1**. The median postoperative follow-up period was 60 months (quartile, 46–86 months). The median age was 68 years (range, 56–77 years), and the median PSA level at initial diagnosis was 8.00 ng/mL (range, 2.13–52.65). D'Amico risk stratification was low in 18 patients (18.6%), intermediate in 40 patients (41.2%), and high in 39 patients (40.2%). Gleason scores at prostatectomy were less than or equal to 6 in 15 patients (15.5%), equal to 7 (3 + 4) in 35 patients (36.1%), equal to 7 (4 + 3) in 33 patients (34.0%), and greater than or equal to 8 in 14 patients (14.4%). Pathological stages were T2 in 69 patients (71.1%), T3a in 21 patients (21.7%), and T3b in seven patients (7.2%). The numbers of patients with lymphatic and peripheral nerve invasion were three (3.1%) and 48 (49.5%), respectively. Additionally, the number of patients with positive resection margins was 47

(48.5%). Overall, 53 patients (54.3%) experienced biochemical recurrence.

Immunohistochemical analysis of SUOX expression

Immunohistochemical analysis of SUOX expression is shown in **Figure 1**. Evaluation of immunostained samples by the two pathologists was highly reliable and reproducible, with intraclass correlation scores for two distinct measurements of 0.837 (excellent). In order to produce objective data, quantitative analysis of SUOX expression was performed with open-source NIH ImageJ software, as described previously [25 26]. A significant correlation was observed between the evaluation using image analysis software and that performed by a pathologist applying the Wilcoxon rank sum test ($p<0.0001$; date not shown). By constructing a receiver operating characteristic curve, SUOX expression was categorized as high (score: ≥ 11) or low (score: ≤ 10). Of these 97 patients, 35 (36.1%) were categorized in the high-expression group (Figure 2a, b), and 62 (63.9%) were categorized in the low-expression group (Figure 2c, d). The correlations between SUOX expression and clinicopathological characteristics are summarized in **Table 2**. PSA level at diagnosis ($p = 0.017$), lymphatic invasion ($p = 0.012$), and biochemical recurrence ($p < 0.0001$) were more frequently identified in the high-expression group than in the low-expression group. However, there were no correlations between SUOX expression and Gleason score ($p = 0.782$).

Identification of SUOX as a biomarker of prostatic biochemical recurrence

Kaplan-Meier curves demonstrated that the time to biochemical recurrence was significantly shorter in patients with high SUOX expression than in those with low SUOX expression ($p = 0.0008$; **Figure 3**). Univariate and multivariate analyses of SUOX expression are shown in **Table 3**. Univariate analysis for time to biochemical recurrence revealed that SUOX expression (high versus low: hazard ratio [HR] = 2.31, 95% confidence interval [CI] = 1.35–4.04, $p = 0.0023$), Gleason score (3 + 4 = 7: HR = 2.93,

95% CI = 1.00–12.4; 4 + 3 = 7: HR = 3.33, 95% CI = 1.13–14.2; ≥ 8: HR = 7.02, 95% CI = 2.15–31.4, p = 0.0103), pathological T stage (T3a: HR = 1.90, 95% CI = 1.01–3.44; T3b: HR = 3.44, 95% CI = 1.27–7.84, p = 0.015), and resection margin positive (HR = 2.60, 95% CI = 1.49–4.67, p = 0.0007) were significant predictors for biochemical recurrence. Moreover, multivariate analysis demonstrated that there were no relationships between biochemical recurrence and Gleason score or pathological T stage. In contrast, SUOX expression level (high versus low: HR = 2.33, 95% CI = 1.32–4.15, p = 0.0037) and resection margin positive (HR = 2.02, 95% CI = 1.08-3.97, p = 0.0283) were identified as independent poor prognostic factor for biochemical recurrence.

Correlation between SUOX expression and Ki-67 labeling index in prostate cancer

Next, we investigated the correlation between SUOX expression and Ki-67 LI in prostate cancer. Immunohistochemical staining revealed high nuclear expression of Ki-67 in tumor cells with strong SUOX expression (Figure. 4a) whereas those with low SUOX expression had only low level of Ki-67 nuclear expression (Figure. 4b). Statistically, the Ki-67 LI was significantly higher in the high SUOX expression group than in the low SUOX expression group (p = 0.0058; Figure. 4c).

Discussion

In this study, we evaluated SUOX staining in resected prostate cancer tissues, and the feasibility of using the results as a predictive factor with respect to biochemical recurrence was considered. Previous reports have considered PSA level at diagnosis, Gleason score and surgical margin status of resected tumor, pathological T stage, and pre-operative PSA as important prognostic factors following radical prostatectomy [27-29]; however, in this study, patients in the SUOX high-expression group exhibited a poor prognosis compared with those in the low-expression group, and the results of univariate and multivariate analyses also suggested that high SUOX expression may be a useful prognostic factor for

biochemical recurrence. The Ki-67 LI in the SUOX high-expression group was significantly higher than that in the low-expression group, and the results indicated that SUOX was related to cell proliferative ability. Based on these findings, histological evaluation of SUOX has been shown to be useful for decision-making regarding the postoperative treatment strategy.

Prostate cancer exhibits high heterogeneity and the proliferation and differentiation potential of prostate cancer cells have been attributed to the involvement of multiple signal transduction and metabolic pathways [30-32]. Although the presence of molecules associated with glucose metabolism has been reported to be correlated with prognosis related to abnormal functions and expression in many malignant tumors [33-35], there have been few reports discussing SUOX as a factor involved in mitochondrial glucose metabolism. SUOX expression in hepatocellular carcinoma decreased in a stepwise manner along with the carcinogenic process, and the expression of SUOX decreased as tumor size increased [22]. In addition, Nakamura et al. found SUOX expression to be diminished in tongue cancer cells compared with healthy tissues, and the expression level continued to decrease as cancerous infiltration progressed [23]. However, a report by Fukushima et al. stated that increases in *SUOX* mRNA in the pancreas were extracted as a poor prognostic factor and that the expression pattern differed depending on the cancer type [36]. In many cancers, hyperglycemia due to the Warburg effect is believed to promote ATP production [37-39]. Recent reports, however, have demonstrated that when cancer cells proliferate in an aerobic metabolic pathway-dependent manner proximal to the tumor vasculature, lactic acid uptake increases in conjunction with activation of the glycolytic system, and cell proliferation is thought to be accelerated as a result of conversion of lactic acid to pyruvate via the activity of lactate dehydrogenase B and subsequent use of pyruvate as a substrate for oxidative phosphorylation [40]. The Ki-67 LI is recognized as a marker of cell proliferation, and Ki-67 LI scores were significantly higher in the SUOX high-expression group than in the low-expression group in this study,

suggesting a relationship with cell proliferative capacity. These findings indicated that cell proliferation may also be induced in prostate cancer as a result of activation of oxidative phosphorylation.

In the present study, SUOX was found to be a prognostic factor, although serum PSA values, Gleason scores, and pathological T factor were not. In general, Gleason score is an important prognostic factor used in routine clinical practice, and prognostic stratification based on the Gleason grade classification system proposed in recent years has been more accurate than classification based on conventional Gleason scores [41 42]. However, in this study, no significant correlation was observed between SUOX expression and Gleason score. Gleason score is a metric used in the histological evaluation of tumor cells. However, it is difficult to characterize the biological properties of tumor cells based on tissue morphology alone. Recent studies have reported that oxidative phosphorylation in the mitochondria of cancer cells is equivalent or greater than that in normal cells [43 44]. This is consistent with the early biochemical recurrence as well as high Ki-67 LI scores observed in the SUOX high-expression group in this study. In these patients, SUOX expression was found to be a promising biomarker for evaluating malignancies that cannot be identified based on tissue morphology alone. Overall, our findings indicate the feasibility of using SUOX expression as a biochemical recurrence factor along with Gleason score.

Although local recurrence and distant metastasis may occur after biochemical recurrence, as a clinicopathological factor, positive resection margin is considered the most important predictor of biochemical recurrence [13-15 45-47]. In fact, in our study, positive resection margin was an independently strong predictor of biochemical recurrence. In addition, high SUOX expression was also demonstrated to serve as a predictor of biochemical recurrence, as reliably as positive surgical margin. In general, biochemical recurrence serves as an indicator of the early stages of relapse [46 47]; the fact that a large number of tumor cells strongly expressing SUOX correlate with tumor

proliferation ability can be hypothesized to suggest that these cells exhibit tumor characteristics that are likely to result in recurrence. Since SUOX expression in resected specimens is as strong a predictor of biochemical recurrence as positive surgical margin is, the extent of SUOX expression could be useful information in determining post-operative treatment strategy. However, SUOX expression may be difficult to assess in small ranges of tissue owing to heterogeneity, as shown in Table 1. As such, in the future, we will measure SUOX expression in prostate biopsy tissues and add this assessment to initial diagnostic tests in order to explore the clinical applications of this marker.

Conclusion

Our findings in this study suggested the superior utility of SUOX immunostaining as a predictor of biochemical recurrence over known prognostic factors in radical prostatectomy cases and highlighted the usefulness of SUOX expression, along with Gleason score and serum PSA value, as a powerful prognostic biomarker for decision-making during postoperative follow-up after total prostatectomy period and for the need for salvage therapy.

Figure legend

Figure 1 Score map of SUOX expression in 97 cases of prostatic cancer.

Figure 2 SUOX expression of prostatic cancer

Photomicrographs of prostatic carcinoma cells stained with hematoxylin-eosin (a, c), or immunostained with SUOX (b, d). SOUX expressions are observed in the cytoplasm. Representative cases of SUOX high and low expression groups are shown in b and d, respectively.

Figure 3 Relationship between SUOX expression and time to biological recurrence. Time to biochemical recurrence was significantly shorter in patients with high SUOX expression than in those with low SUOX expression (p=0.0008).

Figure 4 Comparison of SUOX expression and Ki-67 labeling index in prostate cancer

Immunohistochemical staining revealed high nuclear expression of Ki-67 in tumor cells with strong SUOX expression (Figure. 4a) whereas those with low SUOX expression had only low level of Ki-67 nuclear expression (Figure. 4b). Statistically, the Ki-67 LI was significantly higher in the high SUOX expression group than in the low SUOX expression group (p = 0.0058; Figure. 4c).

References

1. Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. CA: a cancer journal for clinicians 66(1):7-30
2. Nichol AM, Warde P, Bristow RG (2005) Optimal treatment of intermediate-risk prostate carcinoma with radiotherapy: clinical and translational issues. Cancer 104(5):891-905
3. Lowrance WT, Eastham JA, Savage C, Maschino AC, Laudone VP, Dechet CB, Stephenson RA, Scardino PT, Sandhu JS (2012) Contemporary open and robotic radical prostatectomy practice patterns among urologists in the United States. The Journal of urology 187(6):2087-92

4. Hull GW, Rabbani F, Abbas F, Wheeler TM, Kattan MW, Scardino PT (2002) Cancer control with radical prostatectomy alone in 1,000 consecutive patients. The Journal of urology 167(2 Pt 1):528-34
5. Schild SE, Wong WW, Novicki DE, Ferrigni RG, Swanson SK (1996) Detection of residual prostate cancer after radical prostatectomy with the Abbott IMx PSA assay. Urology 47(6):878-81
6. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC (1999) Natural history of progression after PSA elevation following radical prostatectomy. Jama 281(17):1591-7
7. Freedland SJ, Sutter ME, Dorey F, Aronson WJ (2003) Defining the ideal cutpoint for determining PSA recurrence after radical prostatectomy. Prostate-specific antigen. Urology 61(2):365-9
8. D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, Tomaszewski JE, Renshaw AA, Kaplan I, Beard CJ, Wein A (1998) Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. Jama 280(11):969-74
9. D'Amico AV, Chen MH, Roehl KA, Catalona WJ (2004) Preoperative PSA velocity and the risk of death from prostate cancer after radical prostatectomy. The New England journal of medicine 351(2):125-35
10. Horiguchi A, Nakashima J, Horiguchi Y, Nakagawa K, Oya M, Ohigashi T, Marumo K, Murai M (2003) Prediction of extraprostatic cancer by prostate specific antigen density, endorectal MRI, and biopsy Gleason score in clinically localized prostate cancer. The Prostate 56(1):23-9
11. Ohori M, Kattan MW, Koh H, Maru N, Slawin KM, Shariat S, Muramoto M, Reuter VE, Wheeler TM, Scardino PT (2004) Predicting the presence and side of extracapsular extension: a nomogram for staging prostate cancer. The Journal of urology 171(5):1844-9; discussion 49
12. Stephenson AJ, Shariat SF, Zelefsky MJ, Kattan MW, Butler EB, Teh BS, Klein EA, Kupelian PA, Roehrborn CG, Pistenmaa DA, Pacholke HD, Liauw SL, Katz MS, Leibel SA, Scardino PT, Slawin KM (2004) Salvage radiotherapy for recurrent prostate cancer after radical prostatectomy. Jama 291(11):1325-32
13. Swindle P, Eastham JA, Ohori M, Kattan MW, Wheeler T, Maru N, Slawin K, Scardino PT (2008) Do margins matter? The prognostic significance of positive surgical margins in radical prostatectomy specimens. The Journal of urology 179(5 Suppl):S47-51
14. Budaus L, Isbarn H, Eichelberg C, Lughezzani G, Sun M, Perrotte P, Chun FK, Salomon G, Steuber T, Kollermann J, Sauter G, Ahyai SA, Zacharias M, Fisch M, Schlomm T, Haese A, Heinzer H, Huland H, Montorsi F, Graefen M, Karakiewicz PI (2010) Biochemical recurrence after radical prostatectomy: multiplicative interaction between surgical margin status and pathological stage. The Journal of urology 184(4):1341-6
15. Yossepowitch O, Briganti A, Eastham JA, Epstein J, Graefen M, Montironi R, Touijer K (2014) Positive surgical margins after radical prostatectomy: a systematic review and contemporary update. European urology 65(2):303-13

16. Negishi T, Kuroiwa K, Hori Y, Tomoda T, Uchino H, Tokuda N, Furubayashi N, Nagase K, Iwai H, Nakamura M (2017) Predictive factors of late biochemical recurrence after radical prostatectomy. Japanese journal of clinical oncology 47(3):233-38
17. Feng C, Kedia RV, Hazzard JT, Hurley JK, Tollin G, Enemark JH (2002) Effect of solution viscosity on intramolecular electron transfer in sulfite oxidase. Biochemistry 41(18):5816-21
18. Kisker C, Schindelin H, Pacheco A, Wehbi WA, Garrett RM, Rajagopalan KV, Enemark JH, Rees DC (1997) Molecular basis of sulfite oxidase deficiency from the structure of sulfite oxidase. Cell 91(7):973-83
19. D'Errico G, Di Salle A, La Cara F, Rossi M, Cannio R (2006) Identification and characterization of a novel bacterial sulfite oxidase with no heme binding domain from Deinococcus radiodurans. Journal of bacteriology 188(2):694-701
20. Tan WH, Eichler FS, Hoda S, Lee MS, Baris H, Hanley CA, Grant PE, Krishnamoorthy KS, Shih VE (2005) Isolated sulfite oxidase deficiency: a case report with a novel mutation and review of the literature. Pediatrics 116(3):757-66
21. Cohen HJ, Betcher-Lange S, Kessler DL, Rajagopalan KV (1972) Hepatic sulfite oxidase. Congruency in mitochondria of prosthetic groups and activity. The Journal of biological chemistry 247(23):7759-66
22. Jin GZ, Yu WL, Dong H, Zhou WP, Gu YJ, Yu H, Yu H, Lu XY, Xian ZH, Liu YK, Cong WM, Wu MC (2013) SUOX is a promising diagnostic and prognostic biomarker for hepatocellular carcinoma. Journal of hepatology 59(3):510-7
23. Nakamura K, Akiba J, Ogasawara S, Naito Y, Nakayama M, Abe Y, Kusukawa J, Yano H (2018) SUOX is negatively associated with multistep carcinogenesis and proliferation in oral squamous cell carcinoma. Medical molecular morphology 51(2):102-10
24. P.A. H (2016) WHO clasification of tumors of the prostate.138-62
25. Helps SC, Thornton E, Kleinig TJ, Manavis J, Vink R (2012) Automatic nonsubjective estimation of antigen content visualized by immunohistochemistry using color deconvolution. Applied immunohistochemistry & molecular morphology : AIMM 20(1):82-90
26. Ruifrok AC, Johnston DA (2001) Quantification of histochemical staining by color deconvolution. Analytical and quantitative cytology and histology 23(4):291-9
27. Aoun F, Albisinni S, Henriet B, Tombal B, Van Velthoven R, Roumeguere T (2017) Predictive factors associated with biochemical recurrence following radical prostatectomy for pathological T2 prostate cancer with negative surgical margins. Scandinavian journal of urology 51(1):20-26
28. Lee S, Jeong CW, Jeong SJ, Hong SK, Choi W, Byun SS, Lee SE (2013) The prognostic value of pathologic prostate-specific antigen mass ratio in patients with localized prostate cancer with negative surgical resection margins. Urology 82(4):865-9
29. Cao D, Kibel AS, Gao F, Tao Y, Humphrey PA (2010) The Gleason score of tumor at the margin in

radical prostatectomy is predictive of biochemical recurrence. The American journal of surgical pathology 34(7):994-1001

30. Muniyan S, Chen SJ, Lin FF, Wang Z, Mehta PP, Batra SK, Lin MF (2015) ErbB-2 signaling plays a critical role in regulating androgen-sensitive and castration-resistant androgen receptor-positive prostate cancer cells. Cellular signalling 27(11):2261-71
31. Tobiume M, Yamada Y, Nakamura K, Aoki S, Zennami K, Kato Y, Nishikawa G, Yokoi T, Honda N (2011) Significant prognostic factor of immunohistochemical HER-2 expression using initial prostate biopsy specimens with M1b prostate cancer. The Prostate 71(4):385-93
32. Igawa T (2017) Role of protein phosphatases in genitourinary cancers. International journal of urology : official journal of the Japanese Urological Association 24(1):16-24
33. Xiao H, Wang J, Yan W, Cui Y, Chen Z, Gao X, Wen X, Chen J (2018) GLUT1 regulates cell glycolysis and proliferation in prostate cancer. The Prostate 78(2):86-94
34. Reinicke K, Sotomayor P, Cisterna P, Delgado C, Nualart F, Godoy A (2012) Cellular distribution of Glut-1 and Glut-5 in benign and malignant human prostate tissue. Journal of cellular biochemistry 113(2):553-62
35. Vaupel P (2018) Hypoxia in prostate cancer. Journal of Cancer Metastasis and Treatment 4(5)
36. Fukushima H, Yasumoto M, Ogasawara S, Akiba J, Kitasato Y, Nakayama M, Naito Y, Ishida Y, Okabe Y, Yasunaga M, Horiuchi H, Sakamoto E, Itadani H, Mizuarai S, Oie S, Yano H (2016) ARHGEF15 overexpression worsens the prognosis in patients with pancreatic ductal adenocarcinoma through enhancing the motility and proliferative activity of the cancer cells. Molecular cancer 15(1):32
37. Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nature reviews. Cancer 4(11):891-9
38. Liberti MV, Locasale JW (2016) The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci 41(3):211-18
39. Locasale JW, Cantley LC (2011) Metabolic flux and the regulation of mammalian cell growth. Cell metabolism 14(4):443-51
40. Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, De Saedeleer CJ, Kennedy KM, Diepart C, Jordan BF, Kelley MJ, Gallez B, Wahl ML, Feron O, Dewhirst MW (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. The Journal of clinical investigation 118(12):3930-42
41. Epstein JI, Zelefsky MJ, Sjoberg DD, Nelson JB, Egevad L, Magi-Galluzzi C, Vickers AJ, Parwani AV, Reuter VE, Fine SW, Eastham JA, Wiklund P, Han M, Reddy CA, Ciezki JP, Nyberg T, Klein EA (2016) A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. European urology 69(3):428-35
42. Ham WS, Chalfin HJ, Feng Z, Trock BJ, Epstein JI, Cheung C, Humphreys E, Partin AW, Han M (2017)

New Prostate Cancer Grading System Predicts Long-term Survival Following Surgery for Gleason Score 8-10 Prostate Cancer. European urology 71(6):907-12

43. Hensley CT, Faubert B, Yuan Q, Lev-Cohain N, Jin E, Kim J, Jiang L, Ko B, Skelton R, Loudat L, Wodzak M, Klimko C, McMillan E, Butt Y, Ni M, Oliver D, Torrealba J, Malloy CR, Kernstine K, Lenkinski RE, DeBerardinis RJ (2016) Metabolic Heterogeneity in Human Lung Tumors. Cell 164(4):681-94
44. Kerr EM, Gaude E, Turrell FK, Frezza C, Martins CP (2016) Mutant Kras copy number defines metabolic reprogramming and therapeutic susceptibilities. Nature 531(7592):110-3
45. Yossepowitch O, Bjartell A, Eastham JA, Graefen M, Guillonneau BD, Karakiewicz PI, Montironi R, Montorsi F (2009) Positive surgical margins in radical prostatectomy: outlining the problem and its long-term consequences. European urology 55(1):87-99
46. Lattouf JB, Saad F (2003) Digital rectal exam following prostatectomy: is it still necessary with the use of PSA? European urology 43(4):333-6
47. Pound CR, Christens-Barry OW, Gurganus RT, Partin AW, Walsh PC (1999) Digital rectal examination and imaging studies are unnecessary in men with undetectable prostate specific antigen following radical prostatectomy. The Journal of urology 162(4):1337-40

Table 1. Clinicopathological findings of patients characteristic with prostatic cancer

Parameter	
Patients, no	97
Age at diagnosis [year], median (range)	68 (56-77)
PSA level at diagnosis [ng/ml]	
Total, median (range)	8.00 (2.13-52.65)
<10	69 (71.1%)
>10	28 (28.9%)
D'Amico risk stratification, no (%)	
low	18 (18.6%)
intermediate	40 (41.2%)
high	39 (40.2%)
Gleason score, no (%)	
6≦	15 (15.5%)
3+4=7	35 (36.1%)
4+3=7	33 (34.0%)
8≦	14 (14.4%)
Pathological T stage, no (%)	
T2	69 (71.1%)
T3a	21 (21.7%)
T3b	7 (7.2%)
Lymphatic invasion, no (%)	3 (3.1%)
Nerve invasion, no (%)	48 (49.5%)
Resection margin positive, no (%)	47 (48.5%)
Ki-67 positive index (%), no (%)	
1>	70 (72.2%)
1-5	19 (19.6%)
5<	8 (8.2%)
Follow up time [month], median (quartile)	60 (46-86)
Biochemical recurrence, no (%)	53 (54.3%)
Presence	53 (54.3%)
None	44 (45.4%)

no, number

Table 2 : Correlation between SUOX expression and clinicopathological characteristics

Parameter	Low expression of SUOX, no (%)	High expression of SUOX, no (%)	p value
Patients, no	62 (63.9%)	35 (36.1%)	
Age at diagnosis[year], median (range)	68 (55-75)	66 (53-77)	0.079
PSA level at diagnosis[ng/ml]			
Total, median (range)	6.25 (2.13-62.34)	6.91 (4.29-45.29)	0.017
<10	46 (74.2%)	23 (65.7%)	0.379
>10	16 (25.8%)	12 (34.3%)	—
Gleason score			0.782
6≧	11 (17.7%)	4 (11.4%)	—
3+4=7	23 (37.1%)	12 (34.3%)	—
4+3=7	20 (32.2%)	13 (37.1%)	—
8≦	8 (12.9%)	6 (17.1%)	—
Pathological T stage			0.18
T2	48 (77.4%)	21 (60.0%)	—
T3a	10 (16.1%)	11 (31.4%)	—
T3b	4 (6.5%)	3 (8.6%)	—
Presence of lymphatic invasion	0 (0.0%)	3 (8.6%)	0.012
Presence of peripheral nerve invasion	28 (45.2%)	20 (57.1%)	0.256
Resection margin positive	29 (46.8%)	18 (51.4%)	0.659
Biochemical recurrence			<0.0001
Presence	24 (38.7%)	29 (82.9%)	—
None	38 (61.3%)	6 (17.1%)	—

no, number

Table 3 :Univariate and multivariate analysis for time to biochemical recurrence

	Univariate		Multivariate	
Parameter	HR (95% CI)	p value	HR (95% CI)	p value
Age at diagnosis, > 67 [year]	1.22 (0.70-2.15)	0.476		
PSA level at diagnosis, > 10 [ng/ml]	1.72 (0.97-2.97)	0.065		
Gleason score				
6≧	1	0.0103	1	0.0947
3+4=7	2.93 (1.00-12.4)		2.61 (0.88-11.2)	
4+3=7	3.33 (1.13-14.2)		2.64 (0.85-11.6)	
8≦	7.02 (2.15-31.4)		4.78 (1.39-22.1)	
Pathological T stage				
T2	1	0.015	1	0.367
T3a	1.90 (1.01-.344)		1.05 (0.52-2.20)	
T3b	3.44 (1.27-7.84)		1.97 (0.68-5.08)	
Presence of lymphatic invasion	2.27 (0.55-6.23)	0.222		
Presence of peripheral nerve invasion	1.67 (0.97-2.94)	0.062		
Resection margin positive	2.60 (1.49-4.67)	0.0007	2.02 (1.08-3.97)	0.0283
Ki-67 positive index (%)				
1>	1	0.376		
1-5	1.09 (0.57-2.34)			
5<	1.87 (0.71-4.16)			
High expression of SUOX	2.32 (1.35-4.04)	0.0023	2.33 (1.32-4.15)	0.0037

HR, hazard ratio ; CI, confidence interval

Figure 1

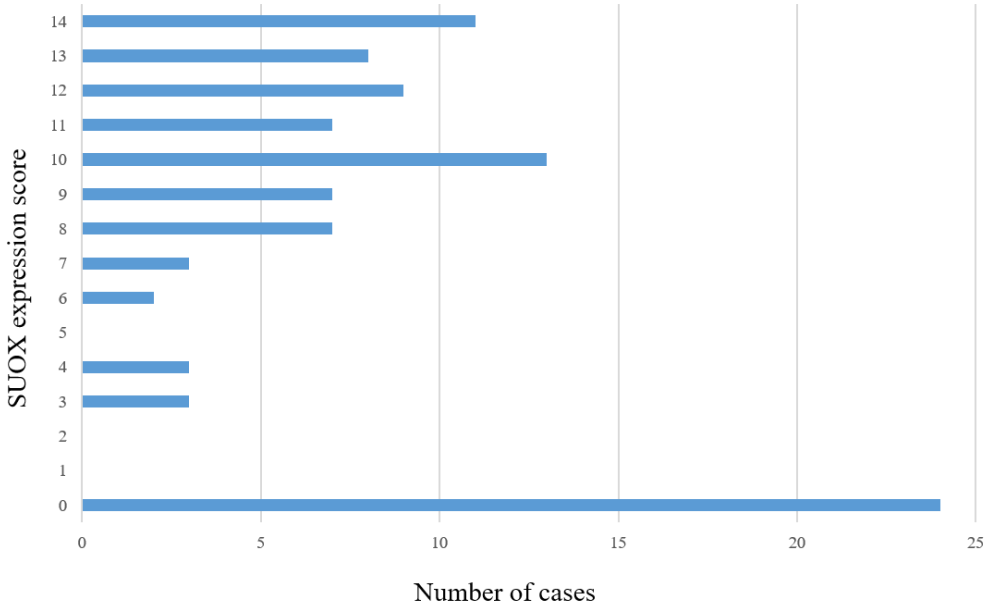

SUOX expression score
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
Number of cases

Figure 2

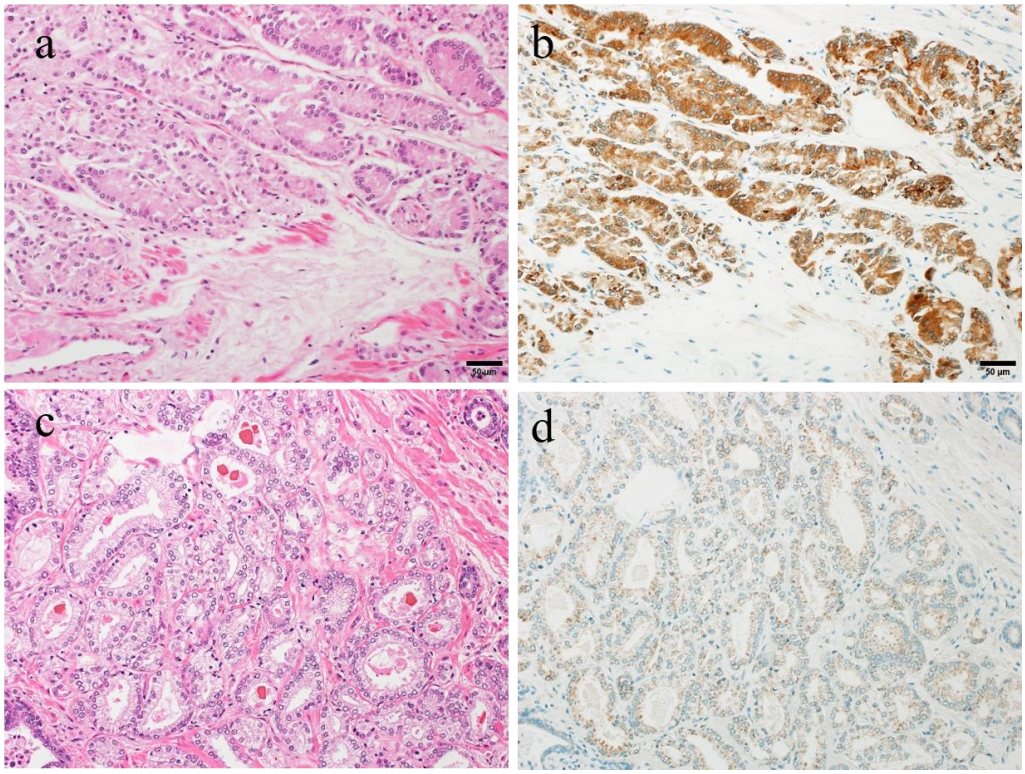


Figure 3

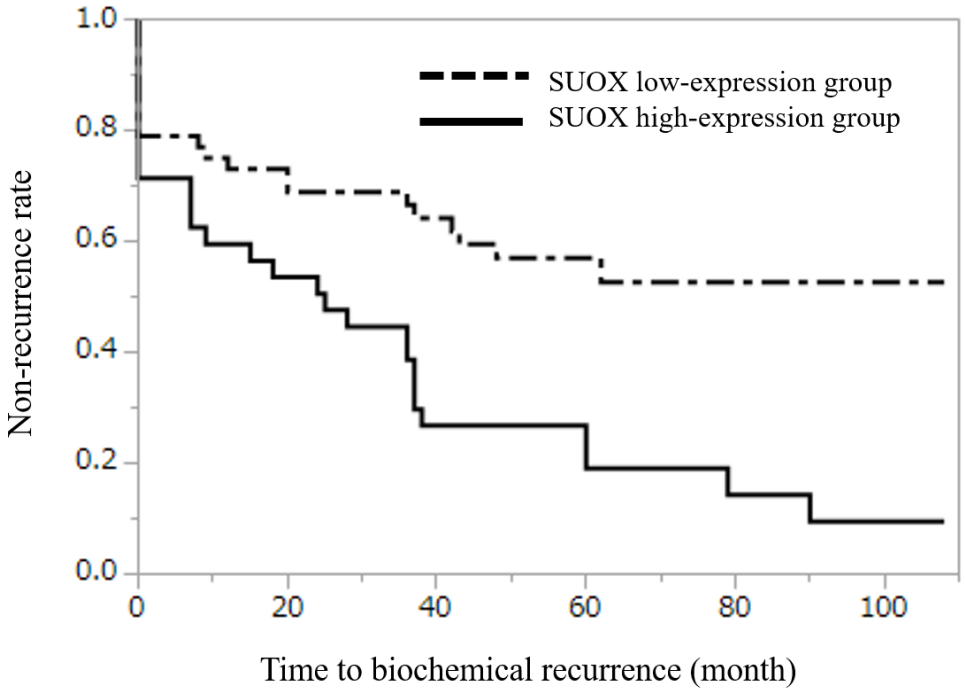
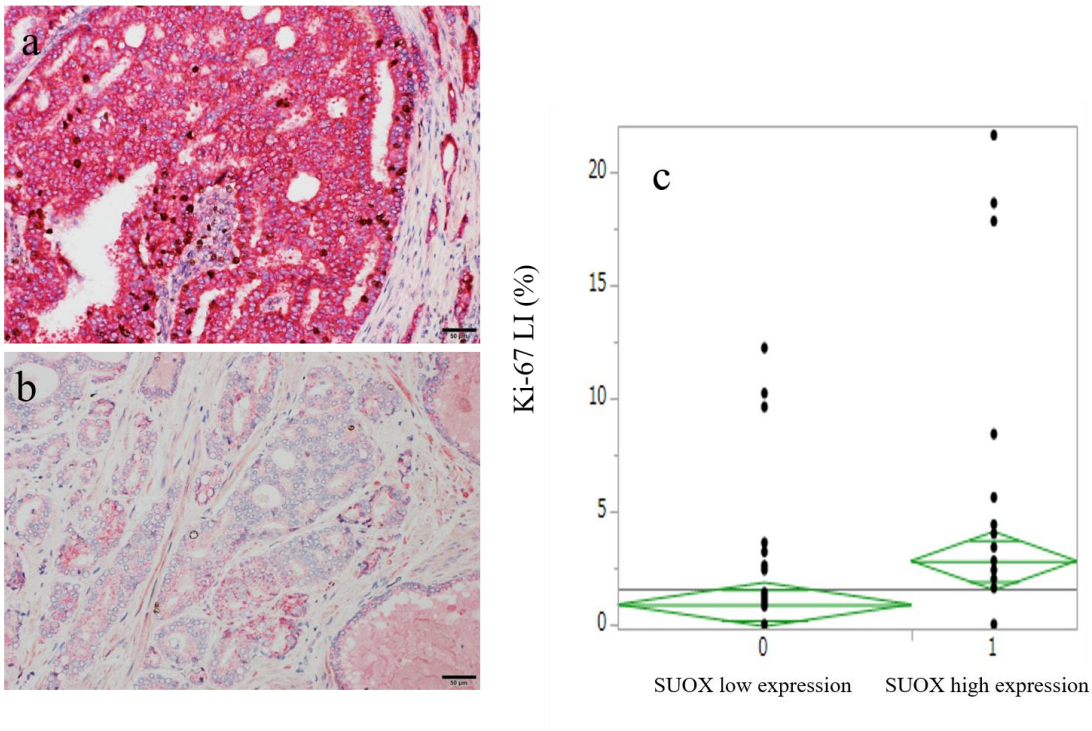

1.0
0.8
0.6
0.4
0.2
0.0
Non-recurrence rate
SUOX low-expression group
SUOX high-expression group
0
20
40
60
80
100
Time to biochemical recurrence (month)

Figure 4


a
b
c
Ki-67 LI (%)
20
15
10
5
0
0
1
SUOX low expression
SUOX high expression