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SIGIRR/TIR8, an important regulator of TLR4 and IL-1R-mediated NF-kB activation, predicts biochemical recurrence after prostatectomy in low-grade prostate carcinomas

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Abstract

Single Ig IL-1-related receptor (SIGIRR) is a negative regulator of toll-like receptor (TLR) 4 and IL-1 mediated activation of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB). The purpose of this study was to qualitatively and quantitatively determine SIGIRR protein expression in human prostate tissues and associate SIGIRR expression with clinical parameters. SIGIRR expression was quantified in glandular prostate tissue using immunohistochemistry and multispectral imaging, and expression was evaluated in relation to clinico-pathological features of benign prostatic hyperplasia (BPH) and prostate cancer (PCa). Subgroupings of low Gleason score (≤6 and 3+4) and high Gleason score (4+3 and ≥8) were used for patient outcomes. SIGIRR was predominantly expressed in the cytoplasm and nucleus of the prostatic epithelium with little expression within the stroma. Compared to normal prostate, cytoplasmic SIGIRR expression was similar in BPH, high-grade prostatic intraepithelial neoplasia (HGPIN), PCa, and metastases. A decrease in nuclear expression was found in metastasis samples (p=0.04). Changes in SIGIRR expression were not associated with Gleason score, pathologic stage, tumor volume, surgical margin status, or serum prostate-specific antigen (PSA; p>0.05). Nuclear (p=0.96) and

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cytoplasmic (p=0.89) SIGIRR expression were not related to patient outcomes in univariable analysis, but in analysis of patients with low Gleason scores, high cytoplasmic SIGIRR expression was associated with biochemical recurrence in both univariable (p=0.01) and multivariable (HR 2.31 [95% CI 1.05–5.06] p=0.04) analysis. Similarly, in multivariable analysis of only low stage (pT2) tumors, SIGIRR independently predicted biochemical recurrence (p=0.009). We conclude that SIGIRR predicts biochemical recurrence in patients with low Gleason score and low pathological stage prostate cancer.

Keywords

SIGIRR; multispectral imaging; NF-κB; biomarker; indolent; prostate cancer

INTRODUCTION

An estimated 220,000 new cases of prostate cancer (PCa) are expected in 2015, and approximately 27,500 men will die due to PCa related causes [1]. Metastatic spread of PCa is the primary cause of mortality, and prognosis is notoriously poor in patients with metastatic PCa, with only one-quarter of men surviving at five years [2]. Physical or chemical castration, with or without radiation, chemotherapy, and immunotherapy, is standard treatment for metastatic PCa and normally results in a tumor regression and decreased serum prostate-specific antigen (PSA) [3, 4]. However, after castration, metastatic tumors inevitably transition to a lethal castration-resistant state (CRPC) and continue proliferating without the presence of androgen. The ability to predict which PCa tumors will progress and metastasize using clinical, pathological, and biological markers is vital in shaping pre- and post-surgical treatment and surveillance regimens for localized PCa.

Currently, common clinical and pathological variables used to predict metastatic recurrence include, but are not limited to, the following: Gleason score, serum prostate-specific antigen levels, surgical margin status, positive lymph node status, extracapsular extension, and seminal vesicle involvement [5, 6]. The association of inflammation with both benign and malignant prostate disease, including prostate cancer prognosis, has been well documented [7–11], but the molecular mechanisms associated with inflammation in the prostate have yet to be fully characterized.

Single Ig IL-1-related receptor (SIGIRR) is a member of the toll/IL-1R (TIR) superfamily and is a negative regulator of the toll-like receptor (TLR) 4 and IL-1R signaling pathways of transcription factor activation, including nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) [12–14]. Because NF-κB is a key orchestrator of cancer-associated inflammation [15], it has been postulated that SIGIRR may be a key regulator of carcinogenesis and metastatic progression of some tumors; indeed, SIGIRR expression is associated with malignant disease in the gastrointestinal tract [16–18] but no SIGIRR studies have been reported in the prostate. With recent reports highlighting the importance of inflammation and NF-κB signaling in the prostate [7, 9, 10, 19], we hypothesized that SIGIRR is a key mediator of prostate tumor progression and metastatic spread. Therefore, the purpose of this study was to assess the expression of SIGIRR protein in prostate tissues

using immunohistochemistry and quantitative multispectral imaging, and to investigate the relationship of SIGIRR expression with metastatic disease progression.

MATERIALS AND METHODS

Patient population and tissue microarray

Formalin-fixed, paraffin-embedded tissues were obtained from the University of Wisconsin Department of Pathology and Laboratory Medicine archive under Institutional Review Board approval. A prostate cancer progression tissue microarray (pTMA) and prostate cancer outcomes tissue microarray (oTMA) were constructed, as described previously [20]. Cores were 0.6mm in diameter and spaced 0.2mm apart both vertically and horizontally using a Manual Tissue Arrayer (Beecher Instruments, Sun Prairie, WI; Model MTA-1). Contained on the pTMA is glandular benign prostatic hyperplasia (BPH) tissue from patients undergoing transurethral resection of the prostate (TURP; 48 cores, in duplicate, from 24 patients), tumor-adjacent normal prostate tissue from prostatectomy (96 cores from 48 patients), high-grade prostatic intraepithelial neoplasia tissue (HGPIN; 50 cores from 25 patients), primary prostate cancer samples (146 cores from 73 patients), and metastatic prostate cancer (44 cores from 22 patients).

The oTMA consists of tumor-adjacent normal prostate tissue (96 cores from 48 patients) and PCa tissue from primary tumors (366 cores from 183 patients). All PCa patients on the oTMA had ≥5 years of regular follow-up and no signs of metastatic disease at time of surgery. Serum PSA nadired to undetectable levels at initial appointment after prostatectomy in all patients, and recurrence was defined as a rise in PSA to ≥0.2 ng/ml and/or local or distant metastatic recurrence within the follow-up period. Both standard and ultrasensitive PSA tests were used due to the timeframe of follow-up. Patients with positive lymph nodes (n=8) were excluded from all analyses. All tissues were inspected by a genitourinary pathologist (WH) at time of TMA construction and clinico-pathological characteristics were defined using American Joint Committee on Cancer (AJCC) 2010 TNM classifications [21].

Immunohistochemistry and automated image analysis

The oTMA and pTMA were sectioned at 5μm and air-dried overnight, and slides were stained by immunohistochemistry as described in previous studies [22–24]. Rabbit polyclonal anti-SIGIRR (Abcam, Cambridge, MA; 1:100 in Renoir Red [Biocare, Concord, MA]) was applied to slides, and goat anti-rabbit Mach 2 (Biocare) horseradish peroxidase-conjugated secondary antibody was then linked to primary anti-SIGIRR antibody. 3,3′-diaminobenzidine (DAB) chromogen (Biocare) was used for detection of SIGIRR. Hematoxylin was used as a counterstain.

Staining was analyzed using the automated Vectra platform (PerkinElmer, Waltham, MA) [20, 22–24]. Briefly, TMA slides were loaded into the Vectra slide scanner (PerkinElmer) and a scanning protocol was created based on TMA size and core distribution. Nuance multispectral image cubes (8-bit) were acquired using the 20x objective lens. A spectral library was created by importing images of two control slides stained with only one chromogen into Nuance 3.0.1 software (PerkinElmer). Multispectral image cubes were

imported into inForm software (PerkinElmer) and staining was unmixed using the spectral library created in Nuance with control slides stained for only one chromogen. A training set of images (18% of cores) was used for tissue and cell segmentation, and the algorithm of differentiation was applied to all cores. SIGIRR expression was then quantified in the nucleus and cytoplasm of the epithelium. Cores with <5% epithelial area or significant folding were eliminated from analysis. Each TMA was analyzed independently using training images selected from that particular TMA alone.

Statistical analysis

Expression of SIGIRR was compared between groups using a Student's t-test or one-way analysis of variance (ANOVA) with Dunnett post hoc test and multiplicity reported p-values for significant factors. Baseline clinical and pathological contingencies stratified by SIGIRR expression were evaluated with Chi-square test or Fisher's exact test. Factors associated with biochemical recurrence were analyzed using univariable Cox proportional hazards regression and Kaplan-Meier method with log-rank statistics. To test independent prognostic ability, a multivariable Cox proportional hazards regression model was constructed. Low-grade disease was defined as patients with Gleason score ≤6 or 3+4, and high-grade disease was defined as patients with Gleason score 4+3 or ≥8. Low stage disease was defined as pT2 tumors. For Kaplan-Meier and multivariable Cox regression outcomes analyses, expression of SIGIRR was divided at the median for all PCa patients for both subcellular compartments, and insignificant effects were removed in stepwise fashion. MedCalc v11.4 (MedCalc Software, Ostend, Belgium) and GraphPad Prism (GraphPad Software Inc, La Jolla, CA) were used for statistical analysis and a two-sided p-value <0.05 was considered significant in all analyses.

RESULTS

SIGIRR protein expression in BPH and prostate cancer progression

Protein expression of SIGIRR was first investigated in normal prostate, glandular BPH, and prostate cancer progression, including metastases, using immunohistochemistry and multispectral imaging (Figure 1). In normal and benign prostate tissue, SIGIRR was found predominantly in the epithelium and localized in a baso-lateral fashion, whereas in cancer SIGIRR was more diffusely localized throughout the cytoplasm of epithelial/carcinoma cells. Little SIGIRR was found in the stroma at any stage. No significant differences in total SIGIRR expression were observed in BPH ($p=0.13$), HGPIN ($p=0.26$), PCa ($p=0.13$), or metastases ($p=0.06$) compared to tumor-adjacent normal prostate tissue (Figure 2A). Tissue compartment-specific SIGIRR expression was then quantified and compared between groups. SIGIRR was primarily localized to the cytoplasm of epithelial cells (4-fold higher than stromal cytoplasmic expression; $p<0.0001$) and to nuclei of epithelial cells (3.5-fold higher than stromal nuclear expression; $p<0.0001$), though some stromal nuclei were positive. Expression of SIGIRR was higher in epithelial nuclei than cytoplasm ($p<0.0001$). Compared to normal prostate tissue, no changes in cytoplasmic epithelial SIGIRR expression were observed in BPH ($p=0.37$), HGPIN ($p=0.20$), PCa ($p=0.40$), or metastases ($p=0.31$; Figure 2B). Similarly, compared to normal prostate, nuclear epithelial SIGIRR expression was similar in BPH ($p=0.07$), HGPIN ($p=0.38$), and PCa ($p=0.06$), but a

significant decrease in expression was observed in metastasis samples (p=0.04; Figure 2C). The nuclear to cytoplasmic ratio (N:C ratio) of SIGIRR in epithelial tissues was similar in all groups (p>0.05), indicating no significant changes in subcellular localization along disease progression.

Evaluation of SIGIRR expression and clinico-pathologic features of prostate cancer

The relationship of subcellular SIGIRR expression with common clinico-pathological features of prostate cancer was evaluated using outcomes results from primary tumor samples (Table 1), as described previously [20, 25]. Margin status was unavailable in 3 of 172 patients, and tumor volume was unavailable in 2 of 172 patients. Changes in epithelial nuclear expression of SIGIRR were not associated with Gleason score (p=0.16), pathologic stage (p=0.51), tumor volume (p=0.51), surgical margins (p=0.88), or pre-operative serum PSA (p=0.92). Similarly, changes in expression of SIGIRR in the cytoplasm of epithelial cells were unrelated to pathologic stage (p=0.29), tumor volume (p=0.26), surgical margins (p=0.55), serum PSA (p=0.91), or Gleason score (p=0.06). No changes in the N:C ratio were observed in any variables investigated (p>0.05). In all, our data suggests that SIGIRR expression is largely unrelated to common clinico-pathological features of prostate cancer. Furthermore, subcellular localization of SIGIRR is similar throughout prostate cancer progression, including metastases.

SIGIRR expression predicts biochemical recurrence in patients with low Gleason score

Univariable Cox proportional hazards regression was used to investigate the relationship of SIGIRR expression and clinico-pathological features with biochemical recurrence after prostatectomy. Variables predictive of biochemical recurrence in univariable analysis include: pathologic stage T3a (hazard ratio 2.99 [95% CI 1.42–6.29] p=0.004) and T3b (6.21 [3.22–11.99] p<0.0001) compared to pT2, Gleason score 4+3 or ≥8 (2.70 [1.53–4.76] p=0.0006) compared to 3+4 or ≤6, and tumor volume 30–39% (3.02 [1.24–7.38] p=0.02) and ≥40% (2.67 [1.08–6.62] p=0.03) compared to tumor volume <10%. Positive surgical margins (p=0.10) and pre-operative serum PSA at initial presentation (p=0.51) were not associated with recurrence in univariable analysis. SIGIRR expression as a continuous variable (per 0.01 OD units) was not associated with recurrence in any subcellular compartment (epithelial cytoplasmic SIGIRR HR 1.01 [0.89–1.15] p=0.89; epithelial nuclear SIGIRR HR 1.00 [0.91–1.11] p=0.96).

To further investigate the association of SIGIRR expression with post-surgical prognosis, Kaplan-Meier analysis was used with division of SIGIRR expression at the median of PCa specimens. Similar to our earlier analysis of mean optical density values, baseline clinical and pathological contingencies were not significantly different when patients were stratified by SIGIRR expression (Supplemental Table 1). When all patients were analyzed, SIGIRR expression was not associated with biochemical recurrence in any subcellular compartment (epithelial nuclear SIGIRR p-value=0.18; epithelial cytoplasmic SIGIRR p-value=0.07; Figure 3A). After evaluation of Kaplan-Meier curves, we noticed a pronounced separation of outcomes starting at approximately three years, and we further hypothesized that SIGIRR expression was associated with prognosis in patients with low-grade but not high-grade disease. Indeed, estimated three-year recurrence-free survival was significantly worse in

patients with high Gleason score (4+3 and ≥8; 63.9%) than patients with low Gleason score (≤6 or 3+4; 85.6%; p=0.0004; data not shown). Patients were separated into low-grade (n=139) and high-grade (n=36) disease and SIGIRR expression was re-analyzed with Kaplan-Meier method. Cytoplasmic expression of SIGIRR above the median in the epithelium was associated with poor prognosis in patients with low-grade PCa (p=0.01; estimated 10 year recurrence-free survival: 61.6% vs. 75.7%; Figure 3B) but not in high-grade PCa (p=0.86; Figure 3C).

To test independent prognostic ability, epithelial cytoplasmic SIGIRR expression was incorporated into a multivariable Cox regression analysis (Table 2). When all patients were analyzed, independent predictors of biochemical recurrence included Gleason score 4+3 or ≥8 (p=0.03) and pathologic stage T3a (p=0.007) or T3b (p=0.0009). Cytoplasmic SIGIRR expression, separated at the median, was not significantly associated with prognosis when all patients were analyzed (p=0.06). We then assessed the cohort of patients with low Gleason score, and independent clinico-pathological prognostic factors included pathologic stage T3b (p=0.02) compared to T2 tumors. High SIGIRR expression (HR 2.31 [95% CI 1.05–5.06] p=0.04) was predictive of biochemical recurrence independent of tumor volume, pathologic stage, and surgical margins in patients with Gleason score ≤6 or 3+4. To confirm the association of SIGIRR expression with poor prognosis in less aggressive prostate carcinomas, another multivariable model was constructed in a cohort of low stage (pT2) tumors (n=139). High SIGIRR expression was independently predictive of recurrence (HR 3.01 [1.33–6.83] p=0.009).

DISCUSSION

SIGIRR is an IL-1R like receptor (ILR) and is part of a superfamily of proteins characterized by the presence of a phylogenetically conserved intracellular toll/IL-1R (TIR) domain [12]. Small differences in structure make SIGIRR functionally distinct from other ILRs containing an extracellular Ig domain in that SIGIRR neither directly binds nor enhances IL-1R signaling [13, 14]. Though SIGIRR has a largely conserved TIR domain, the TIR contains two amino acid substitutions: Ser447 (replaced by Cys222) and Tyr536 (replaced by Leu305) [26]. It has been demonstrated previously that this TIR domain is essential for attenuation of recruitment of important signaling components to both IL-1R and TLR4, and that the TIR domain of SIGIRR alone is sufficient for inhibition of LPS/TLR4 signaling [14]. On the other hand, the extracellular domain of SIGIRR is unique in that it only contains one Ig domain, of which the ligand is currently unknown [27, 28]. This Ig domain prevents heterodimerization of IL-1R and its accessory protein IL-1RAcP [14] and is necessary for inhibition of IL-1R signaling. Due to its role in inhibition of IL-1R heterodimerization and sequestration of intracellular signaling components, SIGIRR is known primarily as negative regulator of the TLR4 and IL-1R-mediated pathways inflammation-induced transcription factor activation [27, 29]. Given the strong association of inflammation with malignant and benign prostatic disease [7–11] and the current dearth of information regarding SIGIRR expression in the prostate, the aim of this study was to characterize SIGIRR expression and localization in human prostate tissues.

Because SIGIRR is known as a tumor suppressor in other cancers and an inhibitor of NF-κB activation, we hypothesized that SIGIRR expression would decrease in proliferative diseases like PCa and BPH [16, 19]. In one recent study, continuous activation of NF-κB in a transgenic IκBα± mouse model was insufficient for malignant transformation, but was sufficient to induce a phenotype consistent with BPH [19]. Furthermore, in ARR_2PB-myc-PAI (Hi-Myc)/IκBα$^{+/-}$ mice, continuous activation of NF-κB resulted in prostatic adenocarcinoma at 3 months vs. 6 months in controls [19]. In colorectal cancer, SIGIRR has been established as a tumor suppressor through studies with SIGIRR-deficient APC$^{+/min}$ mice, which resulted in increased microadenoma formation, hyperactivation of mTOR, and increased NF-κB signaling compared to controls [16]. No current studies, to our knowledge, have investigated the protein expression of SIGIRR in prostate tissues.

Through quantification of immunohistochemical staining using multispectral imaging, we found that cytoplasmic SIGIRR expression was not significantly different between normal prostate tissue and BPH. No changes in cytoplasmic SIGIRR expression were observed in HGPIN, PCa, or metastasis samples. The ubiquitously moderate expression of SIGIRR in all prostate tissues suggests tight regulation and conservation of the TLR4 and IL-1R pathways within the prostate, including in benign and malignant disease states. We then investigated the relationship of SIGIRR expression with clinico-pathological characteristics of PCa using a subset of only PCa tissues, as described previously [20, 25]. We found that SIGIRR expression was largely similar between patients after stratification by Gleason score, pathological stage, surgical margin status, serum PSA, and tumor volume. Our data indicates that SIGIRR is a primarily epithelial derived prostate protein that does not significantly change between normal prostate and BPH and PCa or with PCa progression, with the exception of nuclear expression in metastases, which is reduced compared to normal prostate specimens.

We further investigated the association of epithelial SIGIRR expression with biochemical PSA-recurrence in patients with no evidence of metastatic disease at time of surgery (Nx/N0 and M0). Though no statistically significant association was found, we observed a noticeable separation in outcomes between patients with high and low SIGIRR expression starting at approximately three years. We hypothesized that SIGIRR expression was only related to biochemical recurrence in patients with low grade and stage disease. Indeed, we found that patients with high Gleason score PCa were more likely to experience biochemical recurrence within three years than patients with low Gleason score, indicating that Gleason score may be a confounding variable in our analysis of SIGIRR expression. When we assessed SIGIRR expression in a cohort of low Gleason score patients, we found that high cytoplasmic SIGIRR expression was independently associated with increased biochemical recurrence in multivariable analysis. Similar results were found in low pathological stage tumors.

In light of recent recommendations to stop routine PSA screenings in men [30], it is clear that new molecular, pathologic, and protein markers are needed to identify aggressive vs. indolent PCa. We have identified SIGIRR as a novel biomarker of outcomes specifically in patients with low Gleason score and low stage PCa. The association of high SIGIRR expression with poor prognosis after surgery is certainly intriguing given the established role

of SIGIRR as a tumor suppressor [16, 17], and future research is needed to elucidate the role of SIGIRR in the metastatic spread of prostate cancer. Furthermore, we have identified the IL-1R and TLR4-mediated NF-κB activation pathways as potentially important pathways in metastatic spread of low-grade disease. If SIGIRR is serving its canonical function of IL-1R and TLR4 pathway inhibition in PCa, these findings suggest that suppression of particular pathways of NF-κB activation is important in metastatic spread of low grade PCa. Considering the difficultly of prognosis in patients with low grade disease [31], identifying biomarkers such as SIGIRR in the future will be important in improving patient care.

Limitations of this study include sample size, particularly in patients with high Gleason score and high pathological stage disease. Larger studies on SIGIRR expression and PCa prognosis are needed to confirm that SIGIRR is not associated with recurrence in patients with more aggressive cancers.

CONCLUSIONS

SIGIRR is expressed in the nuclei and cytoplasm of epithelial prostate cells, and nuclear expression is decreased in metastatic samples. High cytoplasmic SIGIRR expression is associated with poor prognosis in low Gleason score and low pathological stage PCa. These findings not only identify SIGIRR as a novel marker of biochemical recurrence in less aggressive PCa, but also highlight the need for future studies on NF-κB signaling pathways in earlier stages of PCa progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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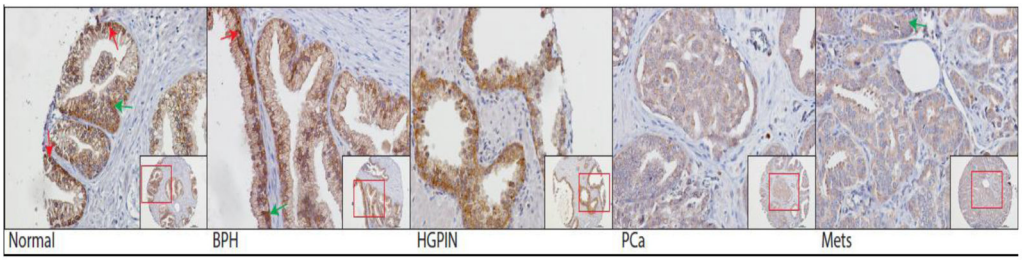

Figure 1.

Localization of SIGIRR in prostate tissues. All benign and cancer tissues were stained for SIGIRR and epithelial expression was quantified using multispectral imaging. Images were acquired using the 20x objective (primary image) or the 10x objective (lower right for each tissue type). Epithelial SIGIRR protein expression was localized to both the cytoplasm, indicated by green arrows, and nuclei, indicated by red arrows, of all prostate tissues. Basal localization was observed in some – but not all – samples, and SIGIRR localization was qualitatively more diffuse in cytoplasm of carcinoma cells than benign prostate cells.

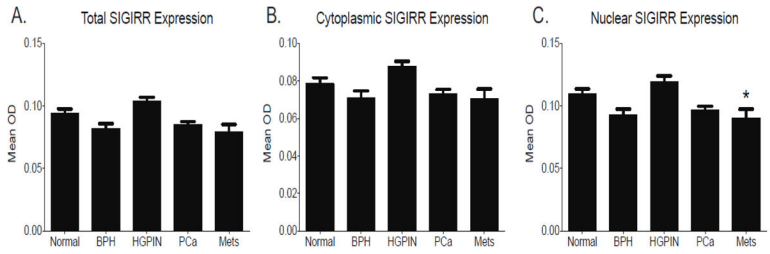

Figure 2.

Protein expression of SIGIRR in human prostate tissues. Compared to normal prostate tissue (average mean OD=0.094; SEM=0.004), total SIGIRR expression was not significantly different in glandular benign prostatic hyperplasia (BPH; 0.082±0.004; p=0.13), high-grade prostatic intraepithelial neoplasia (HGPIN; 0.104±0.004; p=0.26), prostate cancer (PCa; 0.085±0.003; p=0.13), or metastases (Mets; 0.079±0.006; p=0.06). (**A**) Similarly, compared to normal prostate tissue (0.079±0.003), no differences in cytoplasm-specific SIGIRR expression were found in BPH (0.071±0.004; p=0.37), HGPIN (0.088±0.003; p=0.20), prostate cancer (0.073±0.002; p=0.40), or metastases (0.070±0.006; p=0.31). (**B**) Nuclear expression of SIGIRR was similar in BPH (0.093±0.005; p=0.07), HGPIN (0.120±0.005; p=0.38), and prostate cancer (0.097±0.003; p=0.06), compared to normal prostate (0.109±0.005), but a significant decrease in nuclear SIGIRR expression was observed in metastasis samples (0.090±0.007; p=0.04). (**C**)

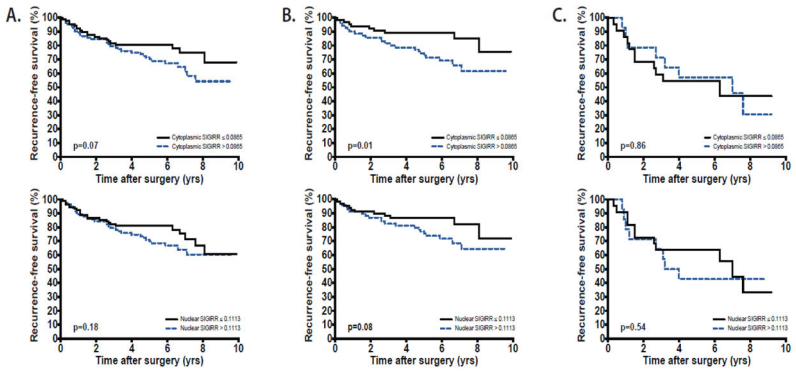

Figure 3.

Kaplan-Meier analysis of the relationship of SIGIRR expression and biochemical recurrence after prostatectomy. Prostate cancer patients on the outcomes tissue microarray were divided at the median of expression of SIGIRR in optical densities units for each of the following subcellular compartments: epithelial cytoplasmic expression (0.0865; top row) and epithelial nuclear expression (median = 0.1113; bottom row). Kaplan-Meier analysis and log-rank statistics were used to evaluate the association between SIGIRR expression and biochemical prostate-specific antigen (PSA)-recurrence after surgery. (**A**) Expression of SIGIRR in all subcellular compartments was not significantly associated with recurrence when all patients were analyzed (p>0.05). (**B**) In sub-analysis of patients with low Gleason score (≤6 and 3+4; n=136), high expression of SIGIRR in the cytoplasm was associated with recurrence (estimated 10 year recurrence-free survival: 61.6% vs. 75.7%; p=0.01). Evaluation of nuclear expression in Kaplan-Meier analysis showed no statistically significant association between SIGIRR expression and recurrence (p=0.08). (**C**) In patients with high Gleason score (4+3 and ≥8; n=36), cytoplasmic (p=0.86) and nuclear (p=0.54) SIGIRR expression were not associated with biochemical recurrence.

Table 1

Relationship of sub-cellular expression of SIGIRR with clinico-pathological features of prostate cancer, average mean OD (±SEM)

	N (%)	Nuclear SIGIRR	Cytoplasmic SIGIRR	SIGIRR N:C ratio
Gleason score				
≤6	73	0.112 (±0.003)	0.092 (±0.002)	1.27 (±0.02)
3+4	63	0.114 (±0.004)	0.087 (±0.006)	1.32 (±0.02)
4+3	14	0.107 (±0.009)	0.088 (±0.006)	1.20 (±0.06)
≥8	22	0.103 (±0.005)	0.080 (±0.004)	1.30 (±0.04)
p-value		0.16	0.06	0.09
Pathologic stage				
T2	139	0.114 (±0.002)	0.089 (±0.002)	1.29 (±0.01)
T3a	18	0.110 (±0.006)	0.090 (±0.004)	1.24 (±0.05)
T3b	15	0.106 (±0.006)	0.080 (±0.004)	1.33 (±0.05)
p-value		0.51	0.29	0.24
Tumor volume				
>10%	39	0.110 (±0.004)	0.088 (±0.004)	1.27 (±0.03)
10–19%	40	0.117 (±0.004)	0.092 (±0.003)	1.27 (±0.03)
20–29%	27	0.118 (±0.006)	0.093 (±0.004)	1.29 (±0.03)
30–39%	32	0.109 (±0.005)	0.084 (±0.003)	1.30 (±0.02)
≥40%	32	0.112 (±0.005)	0.085 (±0.004)	1.33 (±0.03)
p-value		0.51	0.26	0.61
Surgical margins				
Negative	85	0.113 (±0.003)	0.089 (±0.002)	1.27 (±0.02)
Positive	84	0.113 (±0.003)	0.087 (±0.002)	1.30 (±0.02)
p-value		0.88	0.55	0.21
Initial serum PSA				
<5 ng/ml	58	0.114 (±0.004)	0.089 (±0.003)	1.28 (±0.02)
5–10 ng/ml	88	0.113 (±0.003)	0.088 (±0.002)	1.30 (±0.02)
>10 ng/ml	26	0.111 (±0.006)	0.088 (±0.004)	1.26 (±0.03)
p-value		0.92	0.91	0.43

Abbreviations: standard error of the mean (SEM), optical density (OD), prostate-specific antigen (PSA), nuclear to cytoplasm ratio (N:C ratio)

Table 2

Multivariable Cox proportional hazards regression of clinco-pathological features and epithelial cytoplasmic SIGIRR expression

	All patients (n=172)		***Gleason score* ≤6, 3+4 (n=136)**		***Pathologic stage pT2 (n=139)***	
Characteristic	**HR [95% CI]**	**p-value**	**HR [95% CI]**	**p-value**	**HR [95% CI]**	**p-value**
Gleason score						
≤6, 3+4	ref	-	-	-	ref	-
4+3, ≥8	2.10 [1.08–4.09]	0.03	-	-	2.13 [0.87–5.18]	0.10
Tumor volume						
<10%	ref	-	ref	-	ref	-
10–19%	1.01 [0.37–2.75]	0.99	1.45 [0.42–5.06]	0.56	0.94 [0.27–3.21]	0.92
20–29%	1.09 [0.36–3.33]	0.88	1.15 [0.30–4.44]	0.85	0.93 [0.24–3.64]	0.92
30–39%	2.27 [0.89–5.77]	0.09	2.30 [0.69–7.66]	0.18	2.12 [0.68–6.60]	0.20
>39%	1.29 [0.47–3.57]	0.62	1.83 [0.46–7.37]	0.40	1.30 [0.36–4.67]	0.69
Pathologic stage						
T2	ref	-	ref	-	-	-
T3a	2.90 [1.35–6.20]	0.007	2.65 [0.96–7.32]	0.06	-	-
T3b	3.62 [1.70–7.70]	0.0009	3.55 [1.21–10.41]	0.02	-	-
Surgical margins						
Negative	ref	-	ref	-	ref	-
Positive	1.30 [0.68–2.47]	0.42	1.72 [0.76–3.92]	0.20	1.16 [0.55–2.47]	0.70
Epi cyto SIGIRR						
≤0.0865 (median)	ref	-	ref	-		
>0.0865	1.74 [0.99–3.07]	0.06	2.31 [1.05–5.06]	0.04	3.01 [1.33–6.83]	0.009

Abbreviations: hazard ratio (HR), confidence interval (CI), reference (ref), epithelial (epi), cytoplasmic (cyto)