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Nuclear Stat5a/b Predicts Early Recurrence and Prostate Cancer-Specific Death in Patients Treated by Radical Prostatectomy

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Abstract

There is an urgent need for reliable markers to identify patients whose prostate cancer (PCa) will recur after initial therapy and progress to lethal disease. Gleason score (GS) is considered the most accurate predictive marker for disease-specific mortality after primary treatment of localized PCa. The majority of PCas cluster into groups of GS 6 and 7 with considerable variation in the disease recurrence and disease-specific death. In preclinical PCa models, Stat5a/b promotes PCa growth and progression. Stat5a/b is critical for PCa cell viability *in vitro* and for tumor growth *in vivo* and promotes metastatic dissemination of cancer in nude mice. Here, we analyzed the predictive value of high nuclear Stat5a/b protein levels in two cohorts of PCas: Material I (n=562) PCas treated by radical prostatectomy (RP), and Material II (n=106) PCas treated by deferred palliative therapy. In intermediate GS PCas treated by radical prostatectomy, high levels of nuclear Stat5a/b predicted both early recurrence (univariate analysis; $p<0.0001$, multivariate analysis; $HR=1.82$, $p=0.017$) and early PCa-specific death (univariate analysis; $p=0.028$). In addition, high nuclear Stat5a/b predicted early disease recurrence in both univariate ($p<0.0001$) and multivariate ($HR=1.61$;

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No potential conflicts of interest were disclosed.

p=0.012) analysis in the entire cohort of patients treated by RP regardless of the GS. Patients treated by deferred palliative therapy, elevated nuclear Stat5a/b expression was associated with early PCa-specific death by univariate Cox regression analysis (HR=1.59; 95% CI=[1.04, 2.44]; p=0.034). If confirmed in future prospective studies, nuclear Stat5a/b may become a useful independent predictive marker of recurrence of lethal PCa after RP for intermediate GS PCas.

Keywords

Stat5a/b; prostate cancer; recurrence; prostate cancer-specific death; therapy failure

Introduction

A considerable number of surgically treated prostate cancer (PCa) patients experience a biochemical recurrence (1). While biochemical recurrence based on serum PSA elevation is considered as a surrogate marker of PCa mortality, all patients with elevated PSA levels after the initial treatment do not develop clinically significant recurrent disease characterized by distant metastases and castrate-resistant (CR) growth (2). For predicting the course of an individual PCa after the initial treatment, the Gleason grading system(3) is considered the most accurate method. The Gleason score (GS) provides significant predictive information when the value is at the very low or high end of the spectrum(4–6). However, the majority of PCas are of intermediate GS of 6 or 7 with limited prognostic information and, therefore, there is an urgent need for more accurate markers to identify intermediate grade PCas that are likely to recur early and progress to the lethal form of the disease.

Stat5 (Signal Transducer and Activator of Transcription) is a transcription factor that has two highly homologous isoforms, Stat5a and Stat5b, mediating prolactin (7–13), growth hormone and local cytokine/growth factor signaling (14, 15). Inhibition of Stat5a/b causes extensive death across numerous Stat5-positive PCa cell lines and blocks human prostate xenograft subcutaneous and orthotopic tumor growth in nude mice (16–21). Stat5a/b increases nuclear localization and transcriptional activity of androgen receptor (AR) in PCa cells (21, 22). AR, in turn, increases transcriptional activity of Stat5a/b indicating a positive feedback loop between Stat5 and AR (22). While Stat5a/b stimulates AR action in PCa cells, Stat5a/b inhibition induces rapid apoptosis of AR-negative PCa cells as well, indicating that Stat5a/b regulates PCa cell viability not only through the AR but also through AR-independent mechanisms (23). Stat5a/b promotes metastatic behavior of human PCa cells *in vitro* and metastatic progression of PCa *in vivo* in experimental metastases assays (19). Nuclear Stat5a/b is highly expressed in clinical PCas but not in normal prostate epithelium (9, 16). Importantly, the initial tissue microarray analysis of 357 patients treated with radical prostatectomy (RP) combined with various adjuvant therapies indicated that elevated nuclear Stat5a/b is a marker of early recurrence of PCa (24).

Here we evaluated the predictive value of nuclear Stat5a/b in two distinct archival materials of PCas: 1) A homogenous patient cohort of localized primary PCas treated exclusively by RP. Both serum-PSA based biochemical recurrence and PCa-specific death were the end-points. 2) A unique cohort of PCas treated by non-radical deferred therapy with disease-specific survival as the end-point. In summary, the results of these analyses presented here indicate that elevated nuclear Stat5a/b expression predicted early PCa recurrence and early PCa-specific death after RP, especially in intermediate GS PCas.

Materials and Methods

Prostate cancer materials

Archival and de-identified formalin-fixed paraffin-embedded PCa specimens representing two independent clinical materials were analyzed. The use of all tissues was approved by the ethics committee of the respective institutions. Patients whose PCa specimens were uninformative for nuclear Stat5a/b expression did not differ significantly in any clinical characteristics when compared to those with positive or negative Stat5a/b expression. Demographic and clinical characteristics of patients in Materials I and II are presented in Table 1.

Material I

Material I (Table 1) represented PCas treated exclusively by surgical resection of the prostate gland. The PCa samples were obtained from 632 RPs performed as the primary therapy for clinically localized prostate adenocarcinoma in the Turku University Central Hospital during the years 1986–2005. None of the patients had received adjuvant therapy before or immediately after the surgery. The follow-up was conducted by digital rectal examination and post-operative PSA measurement at least three times during the first year after the surgery and at least once a year during the following years. The median post surgery follow-up time was 7.3 years and the median age at surgery was 63 years. The median preoperative PSA level was 8.0 µg/L (0.58 – 290.0 µg/L). Of the 632 patients, 178 (28.1%) had a biochemical disease recurrence defined as elevated serum PSA after surgery. Due to improvement in the sensitivity of the laboratory test over time, the threshold for post-operative biochemical recurrence was serum PSA level of 2.0 µg/L prior to the year 1995 and 1.0 µg/L until the year 1999. After that, a serum PSA level of 0.2 µg/L or above represented recurrence according to the clinical guidelines at the time. The staging of the PCas was performed according to the WHO pTNM classification system (25). Of the patients operated for PCa, 28 (4.4%) died because of the disease during the follow-up, while 44 (7.0%) died of other causes. To construct the tissue microarray (TMA) blocks, three to twelve (median 3.0) adjacent cores (1 mm in diameter) of the predominant carcinoma that was considered the most significant (based on the Gleason grade, the cancer area and/or extra-prostatic extension), were transferred from the donor block to the recipient TMA block. Because of the small amount of tissue in each transferred core, each carcinoma in the TMA was graded according to the predominant Gleason grade pattern. Each patient's original clinical GS was re-evaluated according to the current Gleason grading system (3) criteria using hematoxylin-eosin sections or the original radical prostatectomy specimens. This score was used in the survival analyses and is presented in Table 1. The patients were classified as alive at follow-up, dead from PCa or dead from unrelated causes. The TMA core size was 1.0 mm.

Material II

Material II (Table 1) represented PCas from 419 men with PCa diagnosed at transurethral resection of the prostate (TURP) for obstructive symptoms from 1975 to 1995 at Vasteras Central Hospital in Sweden (26). The TMAs were constructed from resection material from the TURPs (1–8 tumor cores, median 6 cores, from each patient). The treatment of the patients was conservative: If the patient had obstructive symptoms and a palpable tumor, histopathological confirmation of the diagnosis was obtained at TURP, with no previous fine-needle aspiration cytology or core-needle biopsy. None of the patients received hormonal therapy or radiotherapy before TURP. Following TURP, the patients were managed by deferred palliative treatment. Hormonal treatment was given to patients with symptomatic, locally progressive or metastatic disease. The patients were classified as alive

at follow-up, dead from PCa or dead from unrelated causes. The TMA core size was 1.0 mm.

Immunohistochemistry of Stat5a/b

Immunostaining of Stat5a/b was performed as we have demonstrated previously (9, 16, 19, 24). In brief, formalin fixed paraffin embedded 4 µm sections were deparaffinized in xylene, rehydrated and microwave-treated in citrate solution (BioGenex Laboratories, San Ramon, CA) for 20 min. Endogenous peroxidase activity was blocked in 0.3% hydrogen peroxidase and unspecific immunoglobulin binding was blocked by normal goat serum. The monoclonal primary antibody for Stat5a/b (Santa Cruz Biotechnology, Santa Monica, CA) was diluted in 1% bovine serum albumin in PBS (Biogenex, Laboratories) at concentration of 2 µg/mL. Antigen-antibody complexes were detected using biotinylated goat anti-mouse secondary antibody followed by streptavidin-horseradish peroxidase complex using 3,3'-diaminobenzidine as chromogen and Mayers hematoxylin as the counterstain. Lactating mammary gland was used as the positive control tissue (24).

Scoring of nuclear Stat5a/b

Individual prostate tissue microarray cores in both Material I and II were scored for nuclear Stat5a/b levels on a scale from 0 to 3 where 0 represented negative, 1 weak, 2 moderate and 3 strong immunostaining. When combined, the tissue microarrays of Materials I and II contained 1 to 12 (median 3.0) cores per each PCa. The final score for each patient was the highest score of the individual core scores. For example, if a subject had three cores graded as 0, 1, and 3, the final Stat5a/b score for that subject would be 3. Stat5a/b immunostaining scores were available for 562 (88.9%) of 632 patients in Material I and 106 (25%) of 419 patients in Material II. In Material I, the median core scores ranged from 0 to 3 with a median of 1. The range in core scores went from 0 (no difference among core scores) to 3 (at least one core with a 0 score and one with a 3 score) with a median range of 0 and an average range of 0.6. The results were slightly more variable for Material II. The median core scores ranged from 0 to 3 with a median of 1. The range in core scores went from 0 (no difference among core scores) to 3 (at least one core with a 0 score and one with a 3 score) with a median range of 1 and an average range of 1.4.

Statistical Methods

The distribution of biochemical recurrence-free survival (RFS), disease-specific survival (DSS), and overall survival (OS) was estimated using the Kaplan-Meier method. Differences in survival by nuclear Stat5a/b expression, GS, stage, extra-capsular extension, positive surgical margins, seminal vesicle invasion, lymph node involvement and age at diagnosis were evaluated using univariate log-rank test. Association of Stat5a/b nuclear levels with tumor and patient characteristics was evaluated using the Mantel-Haenszel test for trend for categorical outcomes and linear regression for continuous outcomes. Tumor and patient characteristics associated with cumulative survival and nuclear Stat5a/b levels were entered into a Cox proportional hazards regression model. A backwards elimination model-building procedure was used where variables were successively removed from the model until all covariates were statistically significant ($p < 0.15$). In Materials I and II, the survival analysis was performed for the entire cohort and for a subset of intermediate GS tumors (GS 6 or 7 PCa of Gleason grades 3+3, 3+4, or 4+3).

Results

Elevated nuclear Stat5a/b expression predicted increased risk of surgical therapy failure in intermediate Gleason score prostate cancers

To determine whether elevated nuclear Stat5a/b expression would predict outcome of PCa, the levels of nuclear Stat5a/b expression were examined by immunohistochemistry, as described previously (9, 16, 19, 24). PCas with no detectable nuclear Stat5a/b (*top*), weak (score 1, *second from top*), moderate (score 2, *second from bottom*) or strong (score 3, *bottom*) nuclear Stat5a/b expression are presented in Figure 1.

In Material I (Table 1), the patients had been treated exclusively by radical prostatectomy (RP) without neoadjuvant therapies and the samples were retrieved from the surgical specimens. In these patients, nuclear Stat5a/b score positively correlated with most of the clinical tumor characteristics (Table 2). In particular, high Stat5a/b scores (2 and 3) were associated more frequently with extracapsular extension (ECE), high GS, higher preoperative serum PSA levels and greater tumor size (Table 2). Univariate analysis indicated that elevated nuclear Stat5a/b levels were significantly associated with early PSA recurrence in the entire cohort ($p < 0.0001$) (Figure 2A). By Kaplan-Meier estimates, patients without detectable nuclear Stat5a/b expression had recurrence-free survival (RFS) of 72% at 8 years, and patients with a weak nuclear Stat5a/b immunostaining (score 1) had 67% recurrence-free survival at 8 years (Figure 2A). In contrast, patients with nuclear Stat5a/b score of 2 or 3 had an RFS rate of 41% and 42% at 8 years, respectively. This indicates an approximately 30% benefit in RFS at 8 years associated with negative status for nuclear Stat5a/b expression in PCa (Figure 2A). In the univariate log-rank test of the PCa patients of the Material I, all of the known predictive clinical characteristics (GS, pT, ECE, surgical margin status, seminal vesicle invasion and lymph node involvement) were significantly associated with RFS ($p < 0.01$) (data not shown). In a multivariable Cox regression model that took other prognostic markers into account, nuclear Stat5a/b score remained an independent predictor of recurrence-free survival in the entire cohort ($HR = 1.61$; $p = 0.012$) when adjusting for log-transformed tumor size, log-transformed perioperative PSA and pathological stage (Table 3).

In the subset of patients with PCas of intermediate GS of 6 or 7, elevated nuclear Stat5a/b expression predicted significantly shorter RFS after radical prostatectomy ($p < 0.0001$) (Figure 2B). Moreover, in the multivariate analysis confined to patients with GS 6 or 7 PCas, patients without detectable (score 0) or weak (score 1) nuclear Stat5a/b score had significantly lower risk of disease recurrence when compared to patients with nuclear Stat5a/b score 2 or 3 ($HR = 1.82$; $p = 0.017$) (Table 3).

Since increased Stat5a/b expression predicted early PCa recurrence, we next wanted to determine whether levels of nuclear Stat5a/b expression will predict PCa-specific survival after RP (Figures 2C and D). In intermediate GS patients, high nuclear Stat5a/b expression predicted earlier PCa-specific death ($p = 0.028$). Specifically, patients with a nuclear Stat5a/b score of 2 or 3 had lower probability of PCa-specific survival compared with patients whose PCas had nuclear Stat5a/b score of 0 or 1 (Figure 2D). In GS 6 or 7 PCas, there was an approximately 20% benefit in PCa-specific survival at 15 years associated with negative status for nuclear Stat5a/b expression compared to PCas with score 3 nuclear Stat5a/b expression (97% vs. 80%). There were two PCa-specific deaths out of 95 patients (2.1%) with negative Stat5a/b status compared to 5 deaths out of 31 patients (16.1%) with score 3 immunostaining for nuclear Stat5a/b. The small number of deaths due to PCa (28 out of 562 patients) precluded the possibility of a multivariate analysis for PCa-specific survival of the patients in Material I. Collectively, these results suggest that increased nuclear Stat5a/b

levels in PCa predicted early lethal disease-recurrence after radical prostatectomy in Material I.

Given that high nuclear Stat5a/b expression predicted early PCa recurrence and PCa-specific death in patients treated with radical prostatectomy, we analyzed a second material (Material II) which consisted of PCa patients treated with deferred palliative therapy (Table 1). The PCa samples for Stat5a/b analyses were obtained by TURP. Since this group of patients were given no potentially curative treatment, their outcome is close to the natural history of the disease. This is important for identification of patients who would benefit from early aggressive treatments vs. those who could be followed by surveillance regimens. In Material II, known predictive clinical characteristics (GS and T-stage) were associated with PCa-specific survival ($p < 0.0001$) (data not shown). In addition, Stat5a/b score positively correlated with GS ($p = 0.0082$) but was not statistically significantly associated with the tumor stage ($p = 0.25$) (Table 2). Due to the low sample size available for each class of score in this cohort, we divided the Stat5a/b scores into two classes: 0 (i.e., no Stat5a/b immunostaining) vs. 1, 2, 3 (positive nuclear Stat5a/b immunostaining). Intriguingly, nuclear Stat5a/b expression was associated with significantly shorter PCa-specific survival ($p = 0.0453$; Figure 3A) with an approximately 50% benefit at 10 years associated with negative status for nuclear Stat5a/b expression. Of note, among patients with no tissue cores exhibiting nuclear Stat5a/b expression ($n = 10$), no deaths due to PCa were observed. When restricting to PCas of GS of 6 and 7, a similar pattern was observed, but the difference was not statistically significant ($p = 0.5062$; Figure 3B). This is likely due to the fact that the GS 6 and 7 cohort included only 36 patients.

Cox regression analysis was performed to determine the association of Stat5a/b expression with risk of death after adjustment for other prognostic factors. The unadjusted Cox regression analysis confirmed the association found using the log-rank test and indicated that one unit increase in nuclear Stat5a/b expression was associated with a 60% increase in the risk of death (HR=1.59; 95% CI=[1.04, 2.44]; $p = 0.034$). However, after adjustment for GS and T-stage, Stat5 activity was no longer associated with PCa-specific survival (HR=0.99; 95% CI=[0.59, 1.66]; $p = 0.98$). The same analysis was performed on the subset of tumors with GS of 6 and 7. Within this cohort the unadjusted HR was 1.93 (95% CI=[0.52, 7.16]; $p = 0.33$). After adjustment for Tstage and GS, higher nuclear Stat5a/b scores had higher risk of death, although the difference was not statistically significant (HR=1.35; 95% CI=[0.38, 8.20]; $p = 0.65$). In summary, the results from the analyses of PCas treated by deferred therapy indicate that elevated nuclear Stat5a/b expression in untreated PCa was associated with more aggressive PCa behavior and may be useful for prediction of early PCa-specific death.

Discussion

The clinical outcome of PCa is highly variable. During the course of PCa after diagnosis and surgical treatment, the median time for development of metastatic disease is 8 years, after which the median time for cancer-specific death is 5 years (27). However, there are significant differences between individuals in response to therapies and in the clinical course of PCa and, therefore, there is an urgent need for reliable markers to identify PCa patients whose cancer is most likely to recur after the initial therapy and progress to CR and metastatic disease.

The present study demonstrates that high expression of nuclear Stat5a/b in PCa predicts poor response to radical prostatectomy (RP). Specifically, high nuclear Stat5a/b predicted early PCa recurrence and PCa-specific death in patients treated exclusively by RP. In other words, high nuclear Stat5a/b in PCa predicted recurrence of lethal PCa. The findings of the present

study are in accordance with our previous study, which showed that nuclear Stat5a/b predicted early PCa recurrence in 357 PCa patients treated with RP or TURP (24). However, the key limitation of the previous study was the heterogeneous cohort of the patients in terms of the neoadjuvant therapies given, which complicated the interpretation of the data. In the present study, the patients received a single mode of surgical intervention with no neoadjuvant therapies and, therefore, the results of the current study are important for establishing the predictive value of high nuclear Stat5a/b expression in early disease recurrence and cancer-specific death of PCa in response to RP. Our data suggest that PCas treated by RP expressing high nuclear Stat5a/b have increased risk of therapy failure.

In intermediate GS (6 and 7) PCas, high nuclear Stat5a/b expression remained an independent predictive marker of early recurrence and PCa-specific death after RP. Currently, GS is the most frequently used prognostic and predictive factor after PCa diagnosis or surgical treatment where high GS predicts unfavorable clinical outcome (4–6, 28–30). For the clinician responsible for the treatment decisions, the problem with the Gleason scoring criteria is that most of the patients cluster into intermediate GS groups of 6 and 7 where there still is a need for more precise predictive and prognostic factors (5, 31). In addition, there is a considerable inter-observer variation in assessing GS in radical prostatectomy specimens and biopsies, affecting even survival estimates (32, 33). Because of this inter-observer variation, the accuracy of assessing Gleason 3+3, 3+4, 4+3 scores may be compromised and affect the treatment decisions for individual patients. Therefore, there is a factual need for more accurate and reproducible predictive and prognostic markers in order to make more individualized treatment decisions for PCa patients (34). Nuclear Stat5a/b expression levels could thus offer more information on the clinical outcome than Gleason grading alone in routine histological sections.

The third key observation of the current study is that elevated nuclear Stat5a/b protein levels predicted early PCa-specific death in patients treated by palliative deferred treatment. Given that the patients in this cohort were not given any potentially curative treatments, the association of high nuclear Stat5a/b expression with early cancer specific death raises the possibility that Stat5a/b activation is involved in the development of lethal phenotypes of clinical PCa. This concept is supported by our previous findings demonstrating that inhibition of Stat5a/b triggers rapid apoptotic death across numerous Stat5a/b-positive cell lines, and blocks PCa xenograft tumor growth in nude mice (16–19). In addition, nuclear Stat5a/b protein is over-expressed in the majority of distant metastases of clinical PCas, and Stat5a/b promotes metastatic behavior of human PCa cells *in vitro* and *in vivo* (19). Involvement of Stat5a/b in CR growth of PCa is supported by recent data demonstrating transcriptional synergy between Stat5a/b and AR in PCa cells (22), and expression of high levels of nuclear Stat5a/b in clinical CR PCas (21, 22).

Prostate cancer cell clones expressing high levels of nuclear active Stat5a/b may possess a growth advantage in clinical PCas and drive the disease progression. In this study, each PCa analyzed for nuclear Stat5a/b expression was represented by multiple tissue microarray cores (mean 3) taken from the tumor areas constituting the GS. The highest Stat5a/b immunostaining score of the TMA cores was the final nuclear Stat5a/b expression score of each PCa. Intriguingly, the clinical outcome was determined by the presence of even one single tissue microarray core within an individual PCa with high nuclear Stat5a/b protein expression. In other words, if any of the cores within individual PCa showed high level of Stat5a/b expression (immunostaining scores 2 or 3), the likelihood for early PCa recurrence or PCa-specific death was significantly increased. This observation is of particular interest and importance since the finding implies that cancer clones expressing active Stat5a/b may drive histological and clinical disease progression.

In summary, the present study supports the concept that increased Stat5a/b signaling is associated with PCa progression to lethal phenotype, and that high nuclear Stat5a/b expression predicts elevated risk of failure of RP particularly in intermediate GS PCa. The data presented here supports the initiation of prospective studies to determine the clinical utility of nuclear Stat5a/b as a prognostic and predictive marker in PCa.

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References

1. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. *J Urol.* 2004; 172:910–914. [PubMed: 15310996]
2. D'Amico AV, Moul JW, Carroll PR, Sun L, Lubeck D, Chen MH. Surrogate end point for prostate cancer-specific mortality after radical prostatectomy or radiation therapy. *J Natl Cancer Inst.* 2003; 95:1376–1383. [PubMed: 13130113]
3. Epstein JI. An update of the Gleason grading system. *J Urol.* 2010; 183:433–440. [PubMed: 20006878]
4. Albertsen PC, Hanley JA, Fine J. 20-year outcomes following conservative management of clinically localized prostate cancer. *Jama.* 2005; 293:2095–2101. [PubMed: 15870412]
5. Eggener SE, Scardino PT, Walsh PC, Han M, Partin AW, Trock BJ, Feng Z, Wood DP, Eastham JA, Yossepowitch O, Rabah DM, Kattan MW, Yu C, Klein EA, Stephenson AJ. Predicting 15-year prostate cancer specific mortality after radical prostatectomy. *J Urol.* 2011; 185:869–875. [PubMed: 21239008]
6. McNeal JE, Gleason DF. [Gleason's classification of prostatic adenocarcinomas]. *Ann Pathol.* 1991; 11:163–168. [PubMed: 1878086]
7. Ahonen TJ, Harkonen PL, Laine J, Rui H, Martikainen PM, Nevalainen MT. Prolactin is a survival factor for androgen-deprived rat dorsal and lateral prostate epithelium in organ culture. *Endocrinology.* 1999; 140:5412–5421. [PubMed: 10537173]
8. Ahonen TJ, Harkonen PL, Rui H, Nevalainen MT. PRL signal transduction in the epithelial compartment of rat prostate maintained as long-term organ cultures in vitro. *Endocrinology.* 2002; 143:228–238. [PubMed: 11751614]
9. Li H, Ahonen TJ, Alanen K, Xie J, LeBaron MJ, Pretlow TG, Ealley EL, Zhang Y, Nurmi M, Singh B, Martikainen PM, Nevalainen MT. Activation of signal transducer and activator of transcription 5 in human prostate cancer is associated with high histological grade. *Cancer Res.* 2004; 64:4774–4782. [PubMed: 15256446]
10. Nevalainen MT, Valve EM, Ahonen T, Yagi A, Paranko J, Harkonen PL. Androgen-dependent expression of prolactin in rat prostate epithelium in vivo and in organ culture. *Faseb J.* 1997; 11:1297–1307. [PubMed: 9409549]
11. Nevalainen MT, Valve EM, Ingleton PM, Harkonen PL. Expression and hormone regulation of prolactin receptors in rat dorsal and lateral prostate. *Endocrinology.* 1996; 137:3078–3088. [PubMed: 8770934]
12. Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, Harkonen PL. Prolactin and prolactin receptors are expressed and functioning in human prostate. *J Clin Invest.* 1997; 99:618–627. [PubMed: 9045863]
13. Nevalainen MT, Valve EM, Makela SI, Blauer M, Tuohimaa PJ, Harkonen PL. Estrogen and prolactin regulation of rat dorsal and lateral prostate in organ culture. *Endocrinology.* 1991; 129:612–622. [PubMed: 1830268]
14. Darnell JE Jr. STATs and gene regulation. *Science.* 1997; 277:1630–1635. [PubMed: 9287210]

15. Ihle JN, Nosaka T, Thierfelder W, Quelle FW, Shimoda K. Jaks and Stats in cytokine signaling. *Stem Cells*. 1997; 15(Suppl 1):105–111. discussion 112. [PubMed: 9368330]
16. Ahonen TJ, Xie J, LeBaron MJ, Zhu J, Nurmi M, Alanen K, Rui H, Nevalainen MT. Inhibition of transcription factor Stat5 induces cell death of human prostate cancer cells. *J Biol Chem*. 2003; 278:27287–27292. [PubMed: 12719422]
17. Dagvadorj A, Collins S, Jomain JB, Abdulghani J, Karras J, Zellweger T, Li H, Nurmi M, Alanen K, Mirtti T, Visakorpi T, Bubendorf L, Goffin V, Nevalainen MT. Autocrine prolactin promotes prostate cancer cell growth via Janus kinase-2-signal transducer and activator of transcription-5a/b signaling pathway. *Endocrinology*. 2007; 148:3089–3101. [PubMed: 17412813]
18. Dagvadorj A, Kirken RA, Leiby B, Karras J, Nevalainen MT. Transcription factor signal transducer and activator of transcription 5 promotes growth of human prostate cancer cells in vivo. *Clin Cancer Res*. 2008; 14:1317–1324. [PubMed: 18316550]
19. Gu L, Vogiatzi P, Pühr M, Dagvadorj A, Lutz J, Ryder A, Addya S, Fortina P, Cooper C, Leiby B, Dasgupta A, Hyslop T, Bubendorf L, Alanen K, Mirtti T, Nevalainen MT. Stat5 promotes metastatic behavior of human prostate cancer cells in vitro and in vivo. *Endocr Relat Cancer*. 2010; 17:481–493. [PubMed: 20233708]
20. Kazansky AV, Spencer DM, Greenberg NM. Activation of signal transducer and activator of transcription 5 is required for progression of autochthonous prostate cancer: evidence from the transgenic adenocarcinoma of the mouse prostate system. *Cancer Res*. 2003; 63:8757–8762. [PubMed: 14695191]
21. Thomas C, Zoubeidi A, Kuruma H, Fazli L, Lamoureux F, Beraldi E, Monia BP, MacLeod AR, Thuroff JW, Gleave ME. Transcription factor Stat5 knockdown enhances androgen receptor degradation and delays castration-resistant prostate cancer progression in vivo. *Mol Cancer Ther*. 2011; 10:347–359. [PubMed: 21216933]
22. Tan SH, Dagvadorj A, Shen F, Gu L, Liao Z, Abdulghani J, Zhang Y, Gelmann EP, Zellweger T, Culig Z, Visakorpi T, Bubendorf L, Kirken RA, Karras J, Nevalainen MT. Transcription factor Stat5 synergizes with androgen receptor in prostate cancer cells. *Cancer Res*. 2008; 68:236–248. [PubMed: 18172316]
23. Gu L, Dagvadorj A, Lutz J, Leiby B, Bonuccelli G, Lisanti MP, Addya S, Fortina P, Dasgupta A, Hyslop T, Bubendorf L, Nevalainen MT. Transcription factor Stat3 stimulates metastatic behavior of human prostate cancer cells in vivo, whereas Stat5b has a preferential role in the promotion of prostate cancer cell viability and tumor growth. *Am J Pathol*. 2010; 176:1959–1972. [PubMed: 20167868]
24. Li H, Zhang Y, Glass A, Zellweger T, Gehan E, Bubendorf L, Gelmann EP, Nevalainen MT. Activation of signal transducer and activator of transcription-5 in prostate cancer predicts early recurrence. *Clin Cancer Res*. 2005; 11:5863–5868. [PubMed: 16115927]
25. Sobin, LHWK. *TNM Classification of Malignant Tumours*. Wiley-Blackwell; 2009.
26. Hammarsten P, Karalija A, Josefsson A, Rudolfsson SH, Wikstrom P, Egevad L, Granfors T, Stattin P, Bergh A. Low levels of phosphorylated epidermal growth factor receptor in nonmalignant and malignant prostate tissue predict favorable outcome in prostate cancer patients. *Clin Cancer Res*. 2010; 16:1245–1255. [PubMed: 20145160]
27. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. *Jama*. 1999; 281:1591–1597. [PubMed: 10235151]
28. Gleason DF. Classification of prostatic carcinomas. *Cancer Chemother Rep*. 1966; 50:125–128. [PubMed: 5948714]
29. Gleason DF. Histologic grading of prostate cancer: a perspective. *Hum Pathol*. 1992; 23:273–279. [PubMed: 1555838]
30. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol*. 1974; 111:58–64. [PubMed: 4813554]
31. Stark JR, Perner S, Stampfer MJ, Sinnott JA, Finn S, Eisenstein AS, Ma J, Fiorentino M, Kurth T, Loda M, Giovannucci EL, Rubin MA, Mucci LA. Gleason score and lethal prostate cancer: does 3 + 4 = 4 + 3? *J Clin Oncol*. 2009; 27:3459–3464. [PubMed: 19433685]

32. Netto GJ, Eisenberger M, Epstein JI. Interobserver Variability in Histologic Evaluation of Radical Prostatectomy Between Central and Local Pathologists: Findings of TAX 3501 Multinational Clinical Trial. *Urology*. 2011; 77:1155–1160. [PubMed: 21146858]
33. Oyama T, Allsbrook WC Jr, Kurokawa K, Matsuda H, Segawa A, Sano T, Suzuki K, Epstein JI. A comparison of interobserver reproducibility of Gleason grading of prostatic carcinoma in Japan and the United States. *Arch Pathol Lab Med*. 2005; 129:1004–1010. [PubMed: 16048389]
34. Evans CP. Identification of molecular targets in urologic oncology. *World J Urol*. 2009; 27:3–8. [PubMed: 19002690]

Figure 1. Immunohistochemical detection of Stat5a/b in prostate cancer

Paraffinembedded sections of prostate cancer tissue microarrays were immunostained with a monoclonal anti-Stat5a/b antibody and biotin-streptavidin amplified peroxidase antiperoxidase immunodetection. 3,3'-Diaminobenzidine was used as chromogen and Mayer's hematoxylin as counterstain. Individual prostate tissue microarray cores in both Material I and II were scored for nuclear Stat5a/b levels on a scale from 0 to 3, where 0 represented negative (A, *x200* and B, *x400*), 1 weak (C, *x200* and D, *x400*), 2 moderate (E, *x200* and F, *x400*) and 3 strong (G, *x200* and H, *x400*) immunostaining. The final score for each patient was the maximum of the individual core scores.

Figure 2. Elevated nuclear Stat5a/b expression predicts early lethal disease recurrence of prostate cancer after radical prostatectomy (Material I)

Kaplan-Meier analyses indicating that high nuclear Stat5a/b expression predicts shorter recurrence-free survival in all patients regardless of Gleason (G1.) scores ($n = 560$) as well as in intermediate G1. score prostate cancer patients ($n = 379$) (a and b). In addition, high nuclear Stat5a/b expression in prostate cancer after surgical treatment predicted shorter prostate cancer-specific survival (c and d).

Figure 3. Elevated nuclear Stat5a/b expression in prostate cancer predicts shorter prostate cancer specific-survival in patients treated with active surveillance (Material II)

Kaplan-Meier curves for prostate cancer-specific survival according to negative (top curve) or positive (bottom curve) Stat5a/b activation status in all patients regardless of Gl. score (n=106) (a) and in Gl. score 6 and 7 patients (n=36) (b).

Table 1

Characteristics of the prostate cancers in Materials I and II.

Variables	Material I				Material II			
	Total number of prostate cancers (n=632)		Cancers with Stat5a/b expression status (n=562)		Total number of prostate cancers (n=419)		Cancers with Stat5a/b expression status (n=106)	
	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)
Age at radical prostatectomy (Mat. I) or TURP (Mat. II), years	632	63 (40–74)	562	63 (40–74)	419	73.6 (51–95)	106	73.9 (51–88.1)
Follow-up time, years	629	7.3 (0.16–24.7)	562	7.3 (0.16–24.7)	419	5.2 (0–25)	106	5.3 (0.1–18.2)
Preoperative PSA µg/L	584	8.0 (0.58–290.0)	523	8.2 (0.58–290)				
Time to recurrence, years	627	11.4 (8.8,19.4) ^I	560	9.9 (6.8,-) ^I				
Tumor size ²	579	1.5 (1–100)	521	1.5 (1–100)				
Gleason score		n (%)		n (%)		n (%)		n (%)
4	14 (2.2)	7 (1.3)	14 (3.3)	1 (0.9)				
5	49 (7.8)	40 (7.1)	84 (20.1)	28 (26.4)				
6	179 (28.3)	159 (28.3)	110 (26.3)	20 (18.9)				
7	252 (39.9)	232 (41.3)	72 (17.2)	16 (15.1)				
8	83 (13.1)	78 (13.9)	50 (11.9)	13 (12.3)				
9	32 (5.1)	31 (5.5)	68 (16.2)	24 (22.6)				
10	-	-	21 (5.0)	4 (3.7)				
unknown	23 (3.6)	15 (2.7)						
Stage								
			cT1a	73 (17.4)				
pT2	332 (52.5)	284 (50.5)	cT1b	137 (32.7)				
pT3	276 (43.7)	259 (46.1)	cT2	107 (25.5)				
pT4	1 (0.2)	1 (0.2)	cT3	81 (19.3)				
			cT4	13 (3.1)				
unknown	23 (3.6)	18 (3.2)	unknown	8 (1.9)				
Extra-capsular extension								

Variables	Material I		Material II	
	Total number of prostate cancers (n=632)	Cancers with Stat5a/b expression status (n=562)	Total number of prostate cancers (n=419)	Cancers with Stat5a/b expression status (n=106)
Positive surgical margins	Yes	252 (39.9)	237 (42.2)	
	No	353 (55.9)	303 (53.9)	
	Unknown	27 (4.3)	22 (3.9)	
Seminal vesicle invasion	Yes	240 (38.0)	216 (38.4)	
	No	377 (59.7)	337 (60.0)	
	Unknown	15 (2.4)	9 (1.6)	
Lymph node involvement	Yes	84 (13.3)	81 (14.4)	
	No	537 (85.0)	474 (84.3)	
	Unknown	11 (1.7)	7 (1.3)	
Stat5a/b nuclear staining score	Yes	30 (4.8)	29 (5.2)	
	No	588 (93.0)	522 (92.9)	
	Unknown	14 (2.2)	11 (2.0)	
RFS = Recurrence-free survival	0	133 (21.0)	133 (23.7)	10 (9.4)
	1	182 (28.8)	182 (32.4)	24 (22.6)
	2	196 (31.0)	196 (34.9)	51 (48.2)
	3	51 (8.1)	51 (9.1)	21 (19.8)
	Unevaluable	70 (11.1)	-	303 (72.3)

RFS = Recurrence-free survival

¹ 95% confidence interval for median RFS. The upper bound is not defined for prostate cancers with Stat5a/b expression status.

² Tumor size is determined as percentage of tumor in all patient's histological sections

Table 2

Association between the level of nuclear Stat5a/b expression and tumor characteristics of Materials I and II.

		Material I					
Tumor characteristics		Nuclear Stat5a/b score				p-value	
		0 n (%)	1 n (%)	2 n (%)	3 n (%)		
pT	1,2	73 (57.5)	97 (54.8)	90 (47.6)	24 (47.1)	0.057	
	3,4	54 (42.5)	80 (45.2)	99 (52.4)	27 (52.9)		
Extra-capsular extension	No	80 (63.5)	106 (59.9)	93 (50.0)	24 (47.1)	0.0054	
	Yes	46 (36.5)	71 (40.1)	93 (50.0)	27 (52.9)		
Positive Margin	No	87 (66.4)	111 (62.7)	111 (57.2)	28 (54.9)	0.055	
	Yes	44 (33.6)	66 (37.3)	83 (42.8)	23 (45.1)		
Lymph node involvement	No	122 (94.6)	174 (97.8)	178 (92.2)	48 (94.1)	0.28	
	Yes	7 (5.4)	4 (2.3)	15 (7.8)	3 (5.9)		
Seminal vesicle invasion	No	116 (89.2)	153 (85.5)	160 (82.1)	45 (88.2)	0.26	
	Yes	14 (10.8)	26 (14.5)	36 (18.0)	6 (11.8)		
Gleason score	4,5	15 (11.7)	18 (10.3)	10 (5.1)	4 (8.2)	0.0053	
	6	44 (34.4)	47 (26.9)	53 (27.2)	15 (30.6)		
	7	52 (40.6)	77 (44.0)	86 (44.1)	17 (34.7)		
	8,9	17 (13.3)	33 (18.9)	46 (23.6)	13 (26.5)		
Tumor Size <i>I</i>		13.6 (11.8, 15.7)	15.7 (14.0, 17.7)	17.8 (15.8, 20.1)	14.8 (11.4, 19.2)	0.036	
Preoperative PSA <i>I</i>		8.1 (7.1, 9.2)	8.3 (7.4, 9.3)	9.8 (8.8, 10.9)	10.8 (8.6, 13.6)	0.0048	
		Material II					
Tumor characteristics		Nuclear Stat5a/b score				p-value	
		0 n (%)	1 n (%)	2 n (%)	3 n (%)		
cT ²	1a	3 (30.0)	7 (29.2)	13 (26.0)	4 (19.1)	0.25	
	1b	3 (30.0)	7 (29.2)	12 (24.0)	6 (28.6)		
	2	3 (30.0)	2 (8.3)	9 (18.0)	3 (14.3)		
	3,4	1 (10.0)	8 (33.3)	16 (32.0)	8 (38.1)		

		Material I					
Tumor characteristics		Nuclear Stat5a/b score				p-value	
		0 n (%)	1 n (%)	2 n (%)	3 n (%)		
Gleason score	4,5	3 (30.0)	11 (45.8)	13 (25.5)	2 (9.5)	0.0082	
	6	3 (30.3)	4 (16.7)	11 (21.6)	2 (9.5)		
	7	2 (20.0)	3 (12.5)	4 (7.8)	7 (33.3)		
	8,9	2 (20.0)	6 (25.0)	23 (45.1)	10 (47.6)		

Table cell entries represent n and percentage of tumors per various Stat5a/b scores (columns) vs. the tumor characteristics (rows). For each tumor characteristic, percentages add to 100 within each column. P-values are from the Mantel-Haenszel 1 degree of freedom chi-square test for trend.

¹ Table cell entries for Tumor Size and Preoperative PSA are geometric means and 95% confidence intervals. P-value from 1 degree of freedom test for trend in a linear regression model.

² One subject with nuclear Stat5a/b score of 2 had unknown cT.

Multivariable Cox regression analysis for Recurrence Free Survival in prostate cancers of Material I. The results are from the final model after backward elimination model selection.

Table 3

		All Subjects (N=459)			Gleason Grades 3+3, 3+4, 4+3, (N=318)		
		Hazard Ratio	95% CI	p-value	Hazard Ratio	95% CI	p-value
Stat5a/b score	2,3	1.61	(1.11,2.33)	0.012	1.82	(1.11,2.94)	0.017
	0,1	1.00	-		1.00	-	
Tumor Size	One unit increase	1.25	(0.97,1.63)	0.09			
Preoperative PSA	One unit increase	1.59	(1.23,2.05)	0.0004	2.29	(1.69,3.10)	<0.0001
pT	3,4	2.78	(1.85,4.17)	<0.0001	3.45	(2.04,5.88)	<0.0001
	2	1.00	-		1.00	-	