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OBJECTIVE

To test the hypothesis that *FYN*, a member of the SRC family of kinases (SFKs), is up-regulated in prostate cancer, as *FYN* is functionally distinct from other SFKs, and interacts with FAK and paxillin (PXN), regulators of cell morphology and motility.

MATERIALS AND METHODS

Through data-mining in Oncomine (<http://www.oncomine.org>), cell-line profiling with immunoblotting, quantitative reverse transcription and polymerase chain reaction (RT-PCR) and immunohistochemical

analysis, we described *FYN* expression in prostate cancer. The analysis included 32 cases of prostate cancer, nine of prostatic intraepithelial neoplasia (PIN) and 19 normal prostates. Samples were scored for the percentage of stained glands and intensity of staining (from 0 to 3). Each sample was assigned a composite score generated by multiplying percentage and intensity.

RESULTS

Data-mining showed an eight times greater *FYN* expression in prostate cancer than in normal tissue; this was specific to *FYN* and not present for other SFKs. Expression of *FYN* in prostate cancer cell lines (LNCaP, 22Rv1, PC3, DuPro) was detected using quantitative RT-PCR and immunoblotting. Expression of *FYN* and its signalling partners FAK and PXN

was detected in human tissue. Comparing normal with cancer samples, there was a 2.1-fold increase in median composite score for *FYN* ($P < 0.001$) 1.7-fold increase in FAK ($P < 0.001$), and a doubling in PXN ($P < 0.05$). There was a 1.7-fold increase in *FYN* ($P < 0.05$) and a 1.6-fold increase in FAK ($P < 0.01$) in cancer compared with PIN.

CONCLUSIONS

These studies support the hypothesis that *FYN* and its related signalling partners are up-regulated in prostate cancer, and support further investigation into the role of the *FYN* as a therapeutic target.

KEYWORDS

FYN, SRC, prostate cancer, paxillin, FAK

INTRODUCTION

Prostate cancer is the most common cancer affecting American men, accounting for >200 000 new cases of cancer diagnosed in 2008 [1]. While many men have disease that is either amenable to local therapy (surgery or radiation), many will develop metastatic disease. It is this population that is at risk of morbidity and death from both the disease and treatment-related side-effects, such as osteoporosis or cardiovascular events. Despite advances in therapy, >30 000 men are expected to die in 2008 from this disease. These figures have driven an aggressive search for promising molecular targets in prostate cancer. Castration is a highly effective and widely used therapy for men with this disease, but most patients will

progress to a castration-resistant state. This progression is associated with increased morbidity and mortality rates. At present only docetaxel-based chemotherapy has been shown to extend survival for this population of patients. Thus, many therapeutic targets have been proposed and explored. Tyrosine kinases are known to be dysregulated in prostate cancer, and as clinically useable agents have become available, several of these have been studied in prostate cancer, including the epidermal growth factor (EGF) receptor, vascular endothelial growth factor receptor, and B/C raf-kinase, none of which have yet shown significant clinical efficacy. Gene expression profiling of non-receptor tyrosine kinases in prostate cancer has shown that the SRC family is particularly dysregulated in prostate cancer [2].

The SRC-family of kinases (SFKs) is one of the most studied families of proteins in cancer biology. Since the identification and description of the pp60c-SRC, eight other proteins sharing significant structural homology have been identified. The SFKs have long been recognized as overexpressed in several cancers, including prostate cancer. Each member is distinguished by a unique region that specifies its respective binding partners and hence function.

FYN is a 59-kDa member of this family and was one of the first members to be identified. The gene encoding *FYN* is located on chromosome 6q21. The most abundant transcript encodes a protein composed of 537 amino acids with a structure similar to the other SFKs, except for the unique region. Like

other SFKs, FYN is a non-receptor tyrosine kinase that functions downstream of several cell-surface receptors. Its best characterized functions are in neuronal development and T-cell signalling [3], but FYN also induces morphogenic transformation when overexpressed [4]. FYN is recognized as an important mediator of mitogenic signals and as a regulator of cell cycle entry, growth and proliferation. It is also known to mediate integrin interactions and hence cell-cell adhesion. FYN is known to interact with several molecular signals including FAK and paxillin (PXN) [5,6] which might account for the described morphogenic transformation and possibly lend insight into its role in cancer.

In this report we present the first series of studies showing the specific importance of FYN in prostate cancer. Our approach used a combination of both data-mining and tissue microarray (TMA) immunohistochemical (IHC) analysis, showing overexpression of FYN in human prostate cancer.

MATERIALS AND METHODS

The expression of *FYN* in prostate cancer was queried using the Oncomine database (<http://www.oncomine.org>) in February 2008. This is a publicly available database summarizing gene-chip experiments across tissue types [7]. Oncomine provides an infrastructure of data-mining tools to query genes and data sets of interest, and to meta-analyse groups of studies. This database was queried for gene expression data for *FYN*, *SRC*, *YES*, *BLK*, *LCK*, *FGR*, *LYN*, *HCK*, and *YRK*. Studies were included if they compared primary prostate cancers to any of the following: normal or benign epithelium, metastatic prostate cancer, prostatic intraepithelial neoplasia (PIN), BPH or hormone-refractory prostate cancer. The *P* values presented are extracted directly from the Oncomine analysis and have not been repeated manually.

All cell lines used were obtained from the American Type Culture Collection (Manassas, VA, USA). Lines used included standard prostate cancer cell lines: LNCaP, CWR22Rv1, PC3, and DuPro; U87 are malignant astrocytes that were used as a positive control for FYN [8]. Cells were grown according to the supplier's recommendations, in RPMI 1640 with 10% fetal calf serum and penicillin/streptomycin supplement.

All human tissue samples used in the study were obtained from the University of Michigan through an interSPORE collaboration. The use of tissue complied with an institutional review board-approved protocol requiring that all samples were kept anonymous to the primary investigational team.

Tissue was analysed in the form of a TMA, the fabrication of which was described by the University of Michigan group elsewhere [9]. In short, the initial TMA used contained 120 patient specimens planned to have triplicate representation on the TMA; each element was 0.6 mm in diameter. Tissue samples included primary tumour from patients with prostate cancer, with Gleason 6–9 disease, metastatic tumour sites, PIN, proliferative inflammatory atrophy, BPH, prostatic stroma and normal prostate tissue. The identity of patients was withheld from the primary analytical group. Normal glands present on the TMA were taken from patients who had prostatectomy or cystectomy. A patient's sample was only considered useable if represented at least twice on the array.

Commercially available antibodies were used for all immunoblotting and IHC studies. Anti-FYN was obtained from Millipore (Burlington, MA, USA); Anti-FAK was obtained from Invitrogen (Carlsbad, CA, USA); and anti-PXN antibody 5H11 was obtained from Biosource (Invitrogen).

For protein extraction and Western blotting, monolayer cells were grown to 80% confluence then washed in ice-cold PBS. Protein lysates were prepared using lysis buffer (10 mmol/L Tris, pH 7.5, 1 mmol/L β -glycerophosphate, 2 mmol/L DDT, 1 mmol/L EDTA, 150 mmol/L NaCl, 0.5 mmol/L NaF, 2 mmol/L NaVO₄, 0.1% NP40, 10 μ mol/L phenylmethylsulphonyl fluoride, 1% Triton X-100 w/v, 70 units/mL aprotinin, and one Complete Protease Inhibitor Cocktail tablet, Roche, Basel, Switzerland). Cells were scraped and placed on ice after being passed through a 27-G needle and subsequently centrifuged at 11 000 g. Protein was quantified using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA); 20 μ g of protein were subjected to SDS-PAGE and transferred to a HyBond Enhanced Chemiluminescence nitrocellulose membrane (GE Healthcare Biosciences, Piscataway, NJ, USA).

For Western blotting, membranes were blocked at 4 °C overnight in TBS-Tween plus 5% (w/v) non-fat dried milk. After incubation with each antibody diluted in blocking solution for 1 h, the membrane was washed for 10 min in blocking solution and then washed six times for 5 min each in TBS-T. The horseradish peroxidase-conjugated secondary antibody was detected using the Super Signal West Femto Maximum Sensitivity Chemiluminescence Substrate (Pierce) according to the manufacturer's directions. Probed membranes were stripped using Pierce Restore Western Blot Stripping Buffer, washed in TBS-T, and blocked overnight before re-probing. The dilutions of antibodies were: anti-FYN 1:1000, anti FAK 1:1000, anti-PXN 1:500. As a loading control, membranes were probed for actin followed by incubation with a goat antimouse IgM-peroxidase-conjugated secondary antibody (Oncogene Research, Uniondale, NY, USA; 1:20 000 and 1:40 000 dilutions of primary and secondary antibodies, respectively).

RNA from cell lines was extracted using an RNAqueous kit (Ambion, Austin, TX, USA) according to the manufacturer's recommendations. Samples were stored at –80 °C until processed. Customized primers for *FYN* were prepared by Integrated DNA Technologies (Coralville, IA, USA). The left primer was: ATG GAA ACA CAA AAG TAG CCA TAA A; and the right primer: TCT GTG AGT AAG ATT CCA AAA GAC C. Data were calibrated to the expression of glyceraldehyde phosphate dehydrogenase. Quantitative PCR was performed using SYBR Green dye on an ABI 7700 (Applied Biosystems, Foster City, CA, USA).

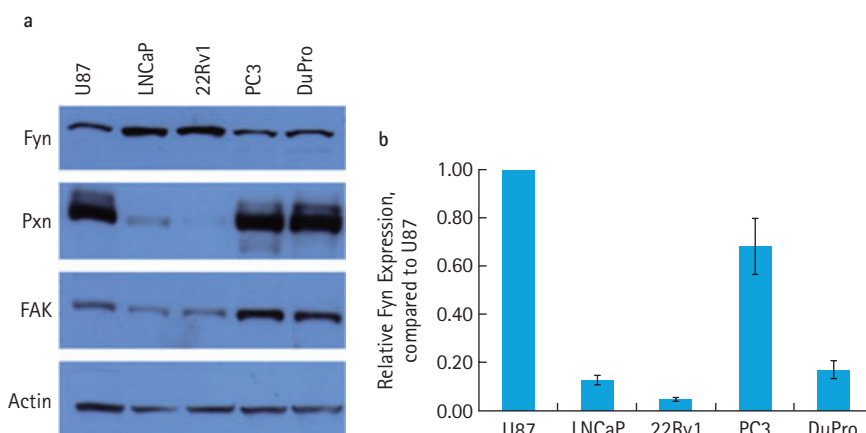
For IHC, stained TMA sections were analysed by a dedicated urological pathologist (H.A.A.) while unaware of sample origin. Results were reported semiquantitatively on a scale of 0–3 for intensity, where 0 was negative, 1 was weak, 2 was moderate and 3 was strong. The percentage of tumour staining was reported as 0–100% in increments of 10%. A composite score was formed using the product of the intensity and percentage of glands staining. Human breast cancer tissue was used as a positive staining control, as recommended by the manufacturer [10]. Human leiomyomas were used as a negative control. FYN was stained using an antibody concentration of 1:50; FAK at 1:100; and paxillin at 1:100.

TABLE 1 Primary FYN antibodies tested, with the results

Manufacturer, id#	Results
Cell Signalling, #4023	Several high molecular weight bands seen in addition to FYN
Abcam, ab32022	Single band ≈59 kDa, but on IHC predominantly nuclear staining.
Upstate, 04-353	Single band at 59 kDa. Cytoplasmic staining on IHC.
Chemicon, MAB8900	No bands seen
Santa Cruz, SC-16	Strong band at 59 kDa but several high molecular weight bands, not specific for IHC

Variable	N or median (range)	TABLE 2 The patient demographics for FYN analysis
Total useable patient samples	86	
Tumour	32	
Gleason 3 + 3	6	
Gleason 3 + 4	8	
Gleason 4 + 3	3	
Gleason 4 + 4	8	
Gleason 4 + 5	7	
Metastases (all sites)	10	
BPH	8	
PIN	9	
Normal prostate	19	
Age, years	64 (43–76)	
Race		
Caucasian	50	
African descent	2	
Other/unknown	34	

FIG. 1. Expression of FYN and signalling partners FAK and PXN in prostate cancer cell lines shown by (a) immunoblotting and (b) quantitative RT-PCR. U87 cells (malignant astrocytes) were used as positive control for FYN expression.



To analyse the TMA data, ANOVA was used to compare expression levels (based on the percentage staining or the composite score) across groups. The equal-variance assumption was verified using Bartlett's test [11]. Post-hoc pair-wise comparisons were performed

with a Bonferroni adjustment for multiple comparisons. The Kruskal–Wallis test was used to compare the ordinal staining intensity score. Also, a nonparametric trend test [12] was used for further examination of expression levels across the naturally ordered

groups. The mean of the duplicate or triplicate samples for each subject was used in the analysis. Statistical significance was indicated at $P < 0.05$.

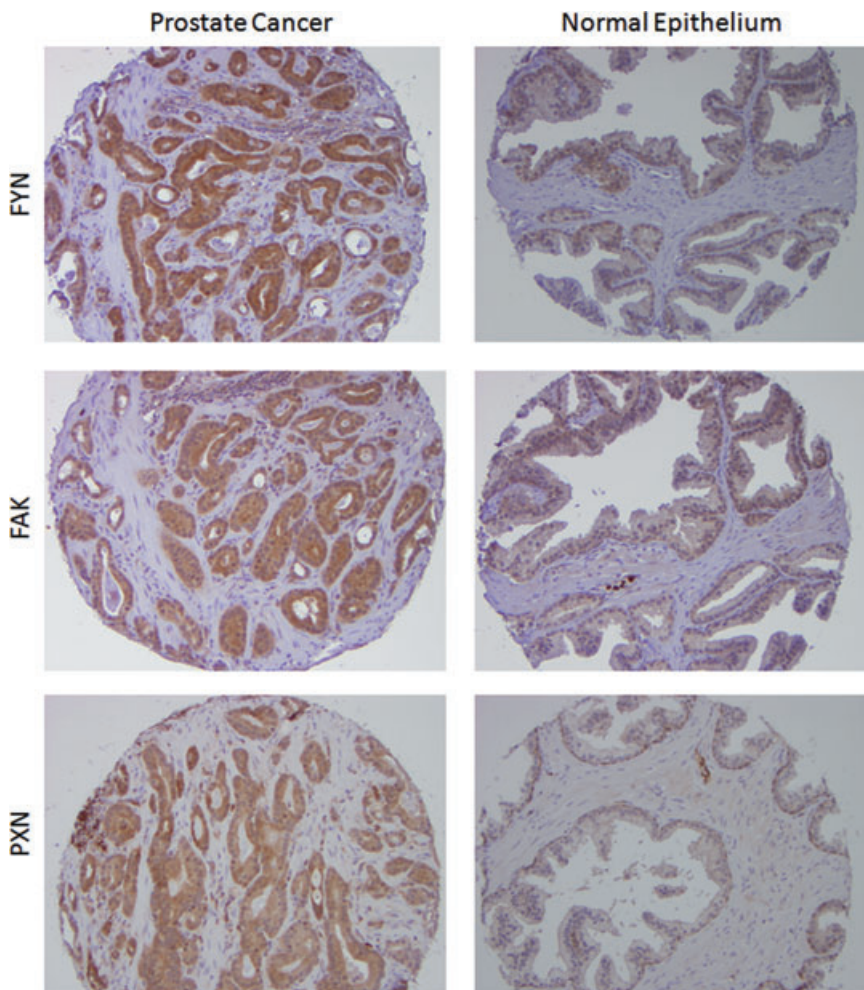
RESULTS

To identify SFKs for analysis, we reviewed available studies in the Oncomine database. On comparing malignant with normal prostate epithelium, the member of this family that arose as the most consistently and strongly overexpressed was FYN, which was eight times greater in cancer ($P < 0.001$) [13]. There was little or no change in the remainder of the SFKs, including LYN, YES, HCK and FGR. The overexpression of FYN further increased by 10 times in the transition from localized to metastatic cancers, while other SFKs were either down-regulated (HCK, LCK) or showed no significant changes in expression (LYN, YES, BLK, or SRC) [14].

FYN was chosen for further investigation as it was identified as the most up-regulated SFK in prostate cancer. Given the homology of the various members of the family, several antibodies were tested and eliminated on the basis of sensitivity and specificity (supplemental data, Table 1). The expression of FYN was evaluated in standard prostate cancer cell lines (Fig. 1a, top). The U87 cell line was used as a positive control, as malignant astrocytes are known to express FYN [8]. Findings were verified by quantitative reverse transcription-PCR (Fig. 1b). There was expression of FYN RNA and protein in all tested cell lines. FYN was not expressed in human leiomyoma samples (immunoblot-verified negative control; data not shown).

We then verified the Oncomine findings in human tissue samples using IHC analysis of a TMA obtained from the University of Michigan, that contained samples of normal prostate, PIN and prostate cancer. There were 86 useable patient samples for the FYN analysis (Table 2). We stained the TMA for total FYN (Fig. 2) and analysed by generating a composite score from the percentage of tumour cells staining and intensity. Several candidate antibodies were tested and discarded (Table 1) if they failed to show sensitivity and specificity to tumour tissues and expected positive control (e.g. lymphocytes) or if the pattern of staining did not correlate with the biology of FYN. For example, an antibody showing predominantly

FIG. 2. Expression of FYN, FAK, and PXN in malignant and non-malignant prostate epithelium. Representative photomicrographs of sections of malignant and non-malignant prostate epithelium.



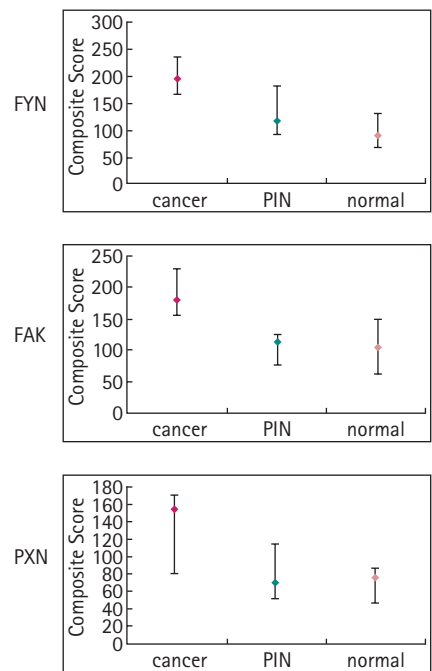
nuclear staining in all samples was declared to be erroneous.

The median (range) composite score for cancer specimens was 200 (23–300); scores did not correlate significantly with Gleason score (data not shown). Of 32 tumour samples, 19 (59%) had scores of 200–300. For normal epithelium the median (range) score was 93 (7–160) and for PIN, 120 (45–220). Figure 3 shows the distribution of composite scores for FYN. Staining of FYN was strong in primary tumour samples compared with non-neoplastic tissue ($P < 0.001$ for the overall comparison). Differences in expression between normal and cancer and PIN and cancer were both statistically significant, based on the composite score. Specifically, there was a 2.1 times greater median composite score in cancer than normal

($P < 0.001$) and a 1.7 times greater score for FYN for cancer than PIN ($P = 0.03$). Furthermore, there was evidence for increasing expression levels across these three naturally ordered groups ($P < 0.001$ for trend). Ten metastatic tumours were represented from various sites (lymph node, lung, liver), with a median (range) scores of 102 (10–290). With so few samples it was not possible to confirm or deny the absence of a trend in FYN expression, but this merits further study.

As FYN interacts with several regulators of cellular morphology and attachment, cell lines and human tissue samples were re-examined for FAK and PXN. Immunoblotting showed co-expression of FAK and PXN with FYN (Fig. 1a, middle, bottom). Both were most highly expressed in the castrate-resistant cell lines (PC3 and DuPro) consistent with the data-

FIG. 3. Plots of (A) FYN (B) FAK and (C) PXN staining in malignant vs non-malignant tissue samples. Composite scores (intensity of staining \times percentage of glandular cells staining) are shown on the Y-axis. The median is plotted with the error bars representing the 25th and 75th percentiles.



mining presented earlier. Castrate-sensitive lines (LNCaP and 22Rv1) showed expression of both FAK and PXN but at a much lower level.

To extend the studies to clinical material, FAK and PXN expression was evaluated on the TMA. Representative sections stained for FAK and PXN are shown in Fig. 2 (middle and bottom). There were 35 useable tumour samples for FAK and 22 for PXN analysis. Our findings for the TMA population are represented graphically in Fig. 3 (middle and bottom).

The median (range) FAK score was 180 (40–300) in tumour samples. There was a tendency for higher Gleason tumours to have higher FAK scores, but this association was not statistically significant. Twelve of 35 (34%) samples had scores of 200–300. In normal epithelium the FAK score was 107 (53–253) and in PIN it was 113 (35–167). In the final analysis, there was 1.7 times greater FAK expression in cancer than normal tissue ($P < 0.001$) and 1.6 times in cancer than PIN

($P < 0.01$). The score in metastatic lesions was 140 (57–290).

The median (range) PXN score for tumour samples was 155 (25–300), with no clear relationship with Gleason score. Only two of 22 (9%) useable specimens had PXN scores of 200–300 (285, 300). The score in normal prostate samples was 77 (25–160) and that for PIN 72 (40–150), but only four samples were available for analysis due to poor transfer. There was a doubling of PXN staining score in cancer over that in normal samples ($P < 0.05$). The few PIN specimens precluded any comparisons between PIN and cancer. These data indicate an up-regulation of FAK and PXN in prostate cancer compared with normal epithelium that correlates with FYN overexpression in cancer.

DISCUSSION

Through a combination of data-mining, immunoblotting and IHC we showed up-regulation of FYN, a particular member of the SRC family of kinases, in prostate cancer. The initial Oncomine queries suggested particularly high overexpression of FYN in cancer compared with normal prostate (non-neoplastic, non-hypertrophic) and *in situ* malignancy (PIN). There was expression of FYN in both a panel of prostate cancer cell lines and human tissue samples. This was accompanied by expression of the FYN signalling partners FAK and PXN, factors known to regulate cellular motility and metastasis. There were discrepancies between the magnitude of FYN measured by quantitative RT-PCR and immunoblot, but there are frequent published reports of discrepancies between RNA and protein expression. Specifically, FYN has been shown to undergo post-transcriptional modification which might affect protein expression [15].

The data-mining further suggested that this up-regulation of expression is specific to FYN and not the other members of the SRC family. While the SRC kinases share similarities in sequence and structure they have differences that might be germane to the development of SFK-directed therapies. Most SFK-directed research in cancer has been aimed at the expression of c-SRC. To date, the role of FYN in cancer biology is relatively unexplored. With >2300 citations in Pubmed referencing the role of SRC and SRC kinases in cancer, there are ≈200 studies mentioning FYN

expression in various cancer models, only a few of which specifically focus on FYN biology. FYN has been implicated as a mediator of EGF-driven transformation of JB6 cells [16]. In breast cancer, FYN expression was shown to correlate with poorer survival, and correlated with FAK up-regulation [17]. In haematological malignancies, FYN has been identified as a putative target for treating BCR-ABL-expressing adult acute lymphoblastic leukaemia, due to the centrality of its relationship to several important molecular signals suspected to drive the proliferation of malignant leukaemic blasts [18]. Compounds active against FYN have shown *in vitro* antiproliferative activity in acute lymphoblastic leukaemia [19]. In other solid tumours such as melanoma, FYN has been implicated as a mediator of integrin signalling, and thus appears to regulate metastatic potential [20].

Interestingly, there is a report of loss of FYN expression in prostate cancer [21]. This group recognized an allelic imbalance at 6q14–22 and sought to identify tumour suppressors associated with this region. They identified FYN as a potential tumour suppressor, noting that the highest levels of FYN were in BPH, compared with malignant tissues which showed little or no FYN expression. While the results appear to be contradictory, the present study does not specifically address the role of FYN in BPH. Members of the SRC family are known to have several different roles in various cellular contexts, and thus it is entirely possible that in one biochemical context FYN serves as a tumour suppressor, while in the altered biochemical landscape of neoplastic transformation (i.e. in the change from pre-invasive, to invasive, then again to metastatic) that FYN serves another role altogether. Further studies will be needed to show the biological role of FYN in these various settings. This type of dynamic signalling behaviour has been seen with other molecular targets (including proposed tumour suppressors) in the setting of prostate cancer [22]. Sørensen *et al.* [21] reported an immunohistochemical analysis similar to that presented here. The present results agree, insofar as there was expression of FYN in normal and hyperplastic epithelium. What requires reconciliation is the absence of FYN staining in tumour tissue as reported by Sørensen *et al.* In the present study, samples from all 32 patients with prostate cancer showed high levels of FYN expression. This might be the result of technical issues, such as

the choice of antibody in the IHC results, as we found during our screening. Finally the study of Sørensen *et al.* suggested that by quantitative PCR there was attenuated expression of FYN in tumour samples from patients. The approach taken made use of whole-tissue homogenates, making the epithelial cell content difficult to control. This is especially important given the congruent findings of absent FYN expression in the stromal compartment.

FYN is positioned downstream of several important cell-surface receptors and upstream of several cellular signals important for prostate cancer progression. Like other SRC family members, it is known to mediate some cell-shape and migration behaviours. As such, its interactions with mediators of cell shape and motility were important factors to study. Our data also suggest that there is an accompanying up-regulation of FAK and PXN, both of which are important regulators of cell shape and interactions with other cells, and the extracellular matrix. Both FAK [23–26] and PXN [26,27] have been recognized as crucial to motility, and thus invasion, which are cellular processes required for metastatic competence and acquisition of the metastatic phenotype.

The expression of FAK and PXN in prostate cancer have been correlated with disease progression [26,28]. FAK has been shown to play a role in prostate cancer metastasis by disrupting integrin-mediated signalling from the extracellular matrix. The invasive ability of DU145 cells on fibronectin was inhibited by silencing FAK expression via siRNA [29]. SRC kinases have been implicated as potential means of modulating FAK activity in prostate cancer and SRC inhibitors have been shown to down-regulate FAK activation [23]. Overexpression of leupaxin, a member of the PXN family, was shown to cause an increase in cellular motility in PC3 cells [30]. Again, SRC kinase inhibitors have been shown to down-regulate the activation of PXN, which in turn results in decreased cellular motility [5]. Given the overexpression of FYN noted here, and the nonspecific nature of most SFK inhibitors, it is likely that the bulk of this effect is mediated by FYN.

These findings gain translational relevance with the introduction of SRC-family inhibitors into clinical practice. Dasatinib is commercially available for treating chronic myelogenous leukaemia, and is currently

being evaluated as a treatment for castrate-resistant prostate cancer. Other agents such as AZD0530 and bosutinib are currently in clinical development, with a host of others to follow. AZD0530, a potent SRC/ABL inhibitor, has been shown to have a potent effect on cellular motility which is SFK-mediated [31]. While labelled as inhibitors of c-SRC, these drugs are known to have various inhibitor effects on cellular tyrosine kinases, including FYN. Furthermore, several inhibitors of both FAK and PXN are currently under development. This raises the potential for combined approaches with these signal-transduction inhibitors in a vertical fashion, which might have potent effects on cellular motility and invasion. If relatively nontoxic, such an approach might be an effective treatment after definitive local therapy in concert with or after castration.

In conclusion, our findings show a statistically significant up-regulation of FYN and its signalling partners FAK and PXN through data-mining, immunoblotting and IHC. It is hoped that further understanding of the role of FYN in prostate cancer development and progression might provide insights into how FYN-inhibitory agents should be used in the clinic. Given our findings, we think that FYN is a promising molecular target for cancer therapeutics.

CONFLICT OF INTEREST

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REFERENCES

- Jemal A, Siegel R, Ward E *et al*. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71–96
- Chang YM, Kung HJ, Evans CP. Nonreceptor tyrosine kinases in prostate cancer. *Neoplasia* 2007; **9**: 90–100
- Resh MD. Fyn, a Src family tyrosine kinase. *Int J Biochem Cell Biol* 1998; **30**: 1159–62
- Kawakami T, Kawakami Y, Aaronson SA, Robbins KC. Acquisition of transforming properties by FYN, a normal SRC-related human gene. *Proc Natl Acad Sci USA* 1988; **85**: 3870–4
- Angelucci A, Schenone S, Gravina GL *et al*. Pyrazolo[3,4-d]pyrimidines c-Src inhibitors reduce epidermal growth factor-induced migration in prostate cancer cells. *Eur J Cancer* 2006; **42**: 2838–45
- Mizutani T, Shiraishi K, Welsh T, Ascoli M. Activation of the lutropin/choriogonadotropin receptor in MA-10 cells leads to the tyrosine phosphorylation of the focal adhesion kinase by a pathway that involves Src family kinases. *Mol Endocrinol* 2006; **20**: 619–30
- Rhodes DR, Kalyana-Sundaram S, Mahavisno V *et al*. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 2007; **9**: 166–80
- Bare DJ, Lauder JM, Wilkie MB, Maness PF. p59fyn in rat brain is localized in developing axonal tracts and subpopulations of adult neurons and glia. *Oncogene* 1993; **8**: 1429–36
- Rubin MA, Mucci NR, Figurski J, Fecko A, Pienta KJ, Day ML. E-cadherin expression in prostate cancer: a broad survey using high-density tissue microarray technology. *Hum Pathol* 2001; **32**: 690–7
- Garcia S, Dales JP, Charafe-Jauffret E *et al*. Poor prognosis in breast carcinomas correlates with increased expression of targetable CD146 and c-Met and with proteomic basal-like phenotype. *Hum Pathol* 2007; **38**: 830–41
- Bartlett M. Properties of sufficiency and statistical tests. *Proc Royal Soc Lond Ser A* 1937; **160**: 268–82
- Cuzick J. A Wilcoxon-type test for trend. *Stat Med* 1985; **4**: 87–90
- Tomlins SA, Mehra R, Rhodes DR *et al*. Integrative molecular concept modeling of prostate cancer progression. *Nat Genet* 2007; **39**: 41–51
- Varambally S, Yu J, Laxman B *et al*. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* 2005; **8**: 393–406
- Lu Z, Ku L, Chen Y, Feng Y. Developmental abnormalities of myelin basic protein expression in fyn knock-out brain reveal a role of Fyn in posttranscriptional regulation. *J Biol Chem* 2005; **280**: 389–95
- He Z, Tang F, Ermakova S *et al*. Fyn is a novel target of (-)-epigallocatechin gallate in the inhibition of JB6 Cl41 cell transformation. *Mol Carcinog* 2008; **47**: 172–83
- Garcia S, Dales JP, Charafe-Jauffret E *et al*. Overexpression of c-Met and of the transducers PI3K, FAK and JAK in breast carcinomas correlates with shorter survival and neoangiogenesis. *Int J Oncol* 2007; **31**: 49–58
- Juric D, Lacayo NJ, Ramsey MC *et al*. Differential gene expression patterns and interaction networks in BCR-ABL-positive and -negative adult acute lymphoblastic leukemias. *J Clin Oncol* 2007; **25**: 1341–9
- Lerma EI, Nguyen VA, Wang T *et al*. Novel compounds with antiproliferative activity against imatinib-resistant cell lines. *Mol Cancer Ther* 2007; **6**: 655–66
- Huang F, Reeves K, Han X *et al*. Identification of candidate molecular markers predicting sensitivity in solid tumors to dasatinib: rationale for patient selection. *Cancer Res* 2007; **67**: 2226–38
- Sorensen KD, Borre M, Orntoft TF, Dyrskjot L, Torring N. Chromosomal deletion, promoter hypermethylation and downregulation of FYN in prostate cancer. *Int J Cancer* 2008; **122**: 509–19
- Lotan TL, Lyon M, Huo D *et al*. Up-regulation of MKK4, MKK6 and MKK7 during prostate cancer progression: an important role for SAPK signalling in prostatic neoplasia. *J Pathol* 2007; **212**: 386–94
- Slack JK, Adams RB, Rovin JD, Bissonette EA, Stoker CE, Parsons JT. Alterations in the focal adhesion kinase/Src signal transduction pathway correlate with increased migratory capacity of prostate carcinoma cells. *Oncogene* 2001; **20**: 1152–63
- Sumitomo M, Shen R, Walburg M *et al*. Neutral endopeptidase inhibits prostate cancer cell migration by blocking focal adhesion kinase signaling. *J Clin Invest* 2000; **106**: 1399–407
- Zheng DQ, Woodard AS, Fornaro M, Tallini G, Languino LR. Prostatic carcinoma cell migration via alpha (v) beta3 integrin is modulated by a focal adhesion kinase pathway. *Cancer Res* 1999; **59**: 1655–64
- Tremblay L, Hauck W, Aprikian AG, Begin LR, Chapdelaine A, Chevalier S. Focal adhesion kinase (pp125FAK) expression, activation and association with paxillin and p50CSK in human metastatic prostate carcinoma. *Int J Cancer* 1996; **68**: 164–71
- Ye L, Lewis-Russell JM, Kynaston H, Jiang WG. Endogenous bone morphogenetic protein-7 controls the motility of prostate cancer cells through regulation of bone morphogenetic protein antagonists. *J Urol* 2007; **178**: 1086–91

- 28 **Rovin JD, Frierson HF Jr, Ledinh W, Parsons JT, Adams RB.** Expression of focal adhesion kinase in normal and pathologic human prostate tissues. *Prostate* 2002; **53**: 124–32
- 29 **Zeng ZZ, Jia Y, Hahn NJ, Markwart SM, Rockwood KF, Livant DL.** Role of focal adhesion kinase and phosphatidylinositol 3'-kinase in integrin fibronectin receptor-mediated, matrix metalloproteinase-1-dependent invasion by metastatic prostate cancer cells. *Cancer Res* 2006; **66**: 8091–9
- 30 **Sahu SN, Nunez S, Bai G, Gupta A.** Interaction of Pyk2 and PTP-PEST with leupaxin in prostate cancer cells. *Am J Physiol Cell Physiol* 2007; **292**: C2288–96
- 31 **Chang YM, Bai L, Yang YC, Kung HJ, Evans CP.** AZD0530 is a novel Src inhibitor with anti-proliferation and anti-migration properties in prostate cancer. *Proc AACR 2007*, Los Angeles, CA, 2007: Abstract #LB-24
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- Abbreviations:** SFK, SRC family kinase; PXN, paxillin; TMA, tissue microarray; IHC, immunohistochemical; PIN, prostatic intraepithelial neoplasia.