

# The interrelationships between Src, Cav-1 and RhoGD12 in transitional cell carcinoma of the bladder

T Qayyum<sup>1</sup>, G Fyffe<sup>1</sup>, M Duncan<sup>1</sup>, PA McArdle<sup>2</sup>, M Hilmy<sup>2</sup>, C Orange<sup>3</sup>, G Halbert<sup>3</sup>, M Seywright<sup>3</sup>, PG Horgan<sup>4</sup>, MA Underwood<sup>2</sup> and J Edwards<sup>\*,1</sup>

<sup>1</sup>Institute of Cancer, College of MVLS, University of Glasgow, Western Infirmary, Glasgow G11 6NT, UK; <sup>2</sup>Department of Urology, Royal Infirmary, Glasgow G4 0SF, UK; <sup>3</sup>Department of Pathology, Western Infirmary, Glasgow G11 6NT, UK; <sup>4</sup>School of Medicine, College of MVLS, University of Glasgow, Royal Infirmary, Glasgow G4 0SF, UK

**BACKGROUND:** The aim of this current study was to assess the expression and activity of Src family kinases, focal adhesion kinase (FAK), caveolin (Cav-1) and RhoGD12 in bladder cancer.

**METHODS:** Fifty-eight patients with a new diagnosis of bladder cancer undergoing transurethral resection were included. Immunohistochemical staining was utilised to assess expression of c-Src, dephosphorylated (Src<sup>Y530</sup>), phosphorylated Src (Y<sup>419</sup>), phosphorylated FAK (FAK<sup>Y861</sup>), Cav-1 and RhoGD12. Expression was assessed using the weighted histoscore method.

**RESULTS:** High expression of dephosphorylated Y<sup>527</sup>, phosphorylated Y<sup>416</sup> and phosphorylated FAK Y<sup>861</sup> in the membrane were associated with increased cancer-specific survival ( $P=0.01$ ,  $P=0.001$ ,  $P=0.008$ , respectively) and expression of Y<sup>416</sup> in the membrane was an independent factor on multivariate analysis when combined with known clinical parameters ( $P=0.008$ , HR = 0.288, 95% CI 0.11–0.72).

**CONCLUSION:** These results demonstrate that in contrast to other solid tumours, activation of the Src family members and downstream signalling proteins are associated with a good prognosis in transitional cell carcinoma of the bladder, and activated Src has a positive relationship with RhoGD12.

*British Journal of Cancer* (2012) **106**, 1187–1195. doi:10.1038/bjc.2012.52 www.bjcancer.com

Published online 21 February 2012

© 2012 Cancer Research UK

**Keywords:** Src kinase; Cav-1; RhoGD12; bladder cancer; survival

Worldwide, there are around 360 000 new cases of bladder cancer diagnosed each year ([www.cancerresearchuk.org](http://www.cancerresearchuk.org)). In the UK alone, bladder cancer is the fifth most common solid non-cutaneous malignancy with approximately 10 000 new cases and 5000 deaths annually ([www.cancerresearchuk.org](http://www.cancerresearchuk.org)). Overall survival is variable; those with non-muscle invasive disease have an 80–90% 5-year survival, whereas in those with muscle invasive disease, the 3-year survival is between 25–50% depending on the degree of invasive disease ([www.cancerresearchuk.org](http://www.cancerresearchuk.org)).

In those with non-muscle invasive disease, the recurrence and progression rate is strongly associated with tumour stage and grade. These factors can be categorised to show the likelihood for recurrence and progression using EORTC risk tables (Sylvester *et al*, 2006). Even in those considered low risk, the 5-year recurrence is 31%, increasing to 78% in those considered to be in the risk high category (Sylvester *et al*, 2006). The 5-year progression rate in those at high risk is 45% (Sylvester *et al*, 2006).

In those with muscle invasive disease undergoing radical cystectomy, the 5-year survival is only 50% (Dalbagni *et al*, 2001; Stein *et al*, 2001; Stein and Skinner, 2006). Despite neoadjuvant chemotherapy in this patient group, published data has only shown a 5% absolute improvement in survival at 5 years (Advanced

Bladder Cancer (ABC) Meta-analysis Collaboration, 2005). The role of neoadjuvant radiotherapy has shown a significant increase in survival in randomised studies (Slack *et al*, 1977; Smith Jr *et al*, 1997). For those with non-resectable disease, palliation of symptoms is the only treatment option.

One potential molecular target is the non-receptor tyrosine kinase Src, the first identified human proto-oncogene. Src kinase has a role in signal transduction of multiple oncogenic cellular processes, including migration, adhesion, invasion, angiogenesis, proliferation and differentiation, and has significant interactions with other cellular proteins such as growth factor receptors (Kopetz *et al*, 2007). The c-Src (commonly referred to as Src) is the prototypical member of the Src kinase family (SFK), with a total of eight members expressed in mammalian cells (c-Src, Blk, Fgr, Fyn, Yes, Hck, Lck and Lyn). Src is composed of a C-terminal tail, kinase domain, two protein–protein interaction domains (SH2, SH3) and a unique amino-terminal domain that varies between the Src family members. Src is activated by a number of pathways. Src kinase activation involves dephosphorylation of a conserved tyrosine residue in the C-terminal negative regulatory tail region (Y<sup>530</sup>) and subsequent autophosphorylation of the Y<sup>419</sup> site in the kinase domain (Cooper and Howell, 1993; Engen *et al*, 2008). Consequently, antibodies to the dephosphorylation of Y<sup>530</sup> and phosphorylation of Src kinase at the Y<sup>419</sup> sites can be used as markers for activated Src kinase (Campbell *et al*, 2008). Activated Src kinase translocates to the cell membrane (Frame, 2002), and therefore, cellular location may also be employed as a surrogate marker of

\*Correspondence: Dr J Edwards; E-mail: joanne.edwards@glasgow.ac.uk  
Received 22 December 2011; revised 23 January 2012; accepted 26 January 2012; published online 21 February 2012

activation (Luo *et al*, 2008). Also, when Src is activated, several downstream signalling proteins such as focal adhesion kinase (FAK) are phosphorylated and could therefore act as biomarkers for Src activation (Nam *et al*, 2005). FAK is phosphorylated at several sites by Src such as Y<sup>397</sup>, Y<sup>576</sup> and Y<sup>577</sup>, but it has been reported that Y<sup>861</sup> is the major site in the carboxyl-terminal domain of FAK (Schaller *et al*, 1994; Calalb *et al*, 1995, 1996). It has been shown that Src expression and activity decreases with bladder tumour stage (Fanning *et al*, 1992; Blaveri *et al*, 2005; Sanchez-Carbayo *et al*, 2006; Wu *et al*, 2009; Thomas *et al*, 2011), although a positive correlation has been shown with breast and prostate cancers (Elsberger *et al*, 2009; Tatarov *et al*, 2009).

Caveolin-1 (Cav-1) is the major coat protein of caveolae, regulating the activity of signalling molecules in cancer (Liu *et al*, 2002), and can make both a negative and positive impact on cancer progression. Cav-1 has been implicated in preventing cell transformation (Galbiati *et al*, 1998) and promoting cell cycle arrest and senescence (Galbiati *et al*, 2001; Volonte *et al*, 2002). Cav-1 expression has been shown to be elevated in various malignancies such as colon (Fine *et al*, 2001), thyroid (Ito *et al*, 2002), lung (Ho *et al*, 2002) and bladder (Fong *et al*, 2003) cancers. In bladder cancer, it has previously been reported that Cav-1 expression is elevated at the onset of oncogenesis (Fong *et al*, 2003) and these levels rise further with progression of the disease in terms of stage and grade (Rajjayabun *et al*, 2001; Fong *et al*, 2003; Kunze *et al*, 2006). Caveolin-1 expression has been demonstrated to be associated with increased cytoplasmic expression in colon cancer but was not associated with tumour stage (Fine *et al*, 2001), whereas membranous expression in ovarian cancer was associated with shorter survival (Davidson *et al*, 2001). Cav-1 was first described as the major substrate for Src in v-Src-transformed cell lines (Glenney Jr and Zokas, 1989; Lee *et al*, 2000), and phosphorylated Cav-1 can either inhibit Src through recruitment of the C-terminal (Li *et al*, 1996; Cao *et al*, 2002) or promote Src activation through an unknown mechanism (Grande-Garcia *et al*, 2007). In addition, high expression of Cav-1 and low expression of Src were associated with metastasis and poor survival in bladder cancer and suggested that these both control bladder metastases through the Rho-ROCK pathway (Thomas *et al*, 2011).

RhoGD12 is known to have a negative and positive impact on cancer. It has previously been demonstrated that RhoGD12 positively correlates with tumour progression and metastases in gastric, ovarian and breast cancers (Tapper *et al*, 2001; Zhang and Zhang, 2006; Cho *et al*, 2009), whereas others have reported it to be a metastatic suppressor in malignancies, including prostate, breast, lung and bladder cancers (Gildea *et al*, 2002; Theodorescu *et al*, 2004; Moon *et al*, 2010; Niu *et al*, 2010). There is controversy in the literature regarding RhoGD12 expression; it has been reported to be both more prominent in the nuclei and cytoplasm of tumour cells (Theodorescu *et al*, 2004; Cho *et al*, 2009). Interestingly, a negative relationship was observed between RNA expression of Src and RhoGD12; it was further suggested that membrane expression of RhoGD12 is elevated when co-transfected with Src Y<sup>527</sup> (Wu *et al*, 2009). Given that Src is still inactive in this state, this would suggest that RhoGD12 expression has a positive relationship with Src inactivity and not Src activity as previously reported.

We hypothesise that it is the activity of Src that drives the relationship with Cav-1 and RhoGD12 expression. The aims of this study were to assess expression of Src, Cav-1 and RhoGD12 in relationship to cancer-specific survival, and to examine the potential interrelationships between these markers.

## PATIENTS AND METHODS

Patients with organ-confined bladder cancer were included for this study. All patients had transitional cell carcinoma. These patients had undergone resection based on investigations in the North

Glasgow NHS Trust. Only those patients were included, whose initial CT scans at the time of diagnosis showed no evidence of regional or metastatic disease, patients who had undergone instillation of intravesical chemotherapy post operation, and whose only subsequent treatment was transurethral resection of recurrences and intravesical therapy. Those patients that subsequently underwent further treatment by way of radical surgery or radiotherapy were excluded. Patients were staged pathologically according to the TNM classification and graded according to the World Health Organisation/International Society of Urological Pathology criteria (Epstein *et al*, 1998). Cancer-specific survival rate was the time from diagnosis until time of death or last follow-up. The cause of death was determined by linkage through the Scottish Cancer Registry. In those who were deceased, if the primary cause of death was of bladder cancer, these were classed as cancer specific, and all other causes were non-cancer specific deaths. The Research Ethics Committee of West of Scotland has approved the study.

Immunohistochemical staining was utilised to assess the expression of c-Src, dephosphorylated Src at Y<sup>530</sup>, phosphorylated Src at Y<sup>419</sup>, FAK at Y<sup>861</sup>, Cav-1 and RhoGD12. Both antibodies for dephosphorylated Src (Y<sup>530</sup>) and phosphorylated Src (Y<sup>419</sup>) are not specific for c-Src because of sequence homogeneity between family members, and can therefore also detect other family members, including Fyn, Yes and Fgr.

Src kinase and activated Src kinase expression (c-Src and Src Y<sup>419</sup>) were investigated using antibodies for c-Src (36D10, Cell Signalling Technology, Beverly, MA, USA) and Y<sup>416</sup>Src (Cell Signalling Technology). Dephosphorylated Src and phosphorylated FAK were investigated using antibodies for Src dephosphorylated at Y<sup>527</sup> (dephosphorylated Y<sup>527</sup>) and FAK Y<sup>861</sup> (Invitrogen, Paisley, UK). In humans, the activated phosphorylations that were investigated in the current study are amino acids Y<sup>530</sup> and Y<sup>419</sup>. Antibodies used relate to the rabbit sequence and not the human sequence. Expression for Cav-1 and RhoGD12 were investigated using an anti-Caveolin 1 antibody (Abcam, Cambridge, UK) and D4-GDI (Spring Bioscience, Pleasanton, CA, USA), respectively.

Tissue sections were dewaxed and rehydrated through graded alcohol. Antigen retrieval was performed by heating tissue sections under pressure for 5 min in a pressure cooker, using citrate buffer pH 6 for c-Src, dephosphorylated Y<sup>527</sup>, FAK Y<sup>861</sup>, Cav-1, RhoGD12 and EDTA buffer pH 9 for Src Y<sup>416</sup>. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). To reduce non-specific binding, the tissue sections were then incubated with 5% normal horse serum (Vector Laboratories, Burlingame, CA, USA) in antibody diluent (DAKO Cytomation, Glostrup, Denmark) for 20 min at room temperature. Incubation with primary antibody was performed with c-Src (1:200) for 60 min at room temperature and overnight at 4 °C for antibodies for dephosphorylated Src Y<sup>527</sup> (1:3000), phosphorylated Src Y<sup>416</sup> (1:25), FAK Y<sup>861</sup> (1:200), Cav-1 (1:500) and RhoGD12 (1:200).

Signal was amplified and visualised using the DAKO Envision Kit (DAKO Cytomation) and the chromagen 3,3'-diaminobenzidine (Vector Laboratories).

Sections were counterstained, dehydrated and mounted. In each run, a positive and negative isotype-matched control was included to ensure no false-positive staining.

## Scoring

Protein expression was assessed using the weighted histoscore method (H score method; Kirkegaard *et al*, 2006). The weighted histoscore grades staining intensity as negative (0), weak (1), moderate (2) and strong (3), then multiplication of the percentage of tumour cells within each category. Agreement between observers was excellent and measured in interclass correlation coefficient, respectively.

Statistical analysis was undertaken using SPSS (Chicago, IL, USA). Cancer-specific survival rates were generated using the

Kaplan–Meier method. The log rank test was utilised to compare significant differences between subset groups using univariate analysis. Multivariate analysis was carried out based on the results of the univariate analysis. Multivariate Cox regression analysis was performed to identify those factors that were independently associated with cancer-specific death. A stepwise backward procedure was utilised to ascertain which of the variables had a significant independent relationship with survival. A  $\chi^2$ -analysis was utilised to assess relationships between pathological parameters and the biomarkers at the various locations. Pearson's correlation was utilised to assess if relationships could be identified between the various proteins at the various cellular locations. *P*-values <0.0003 were deemed significant according to Bonferroni's correction.

**RESULTS**

Analysis was based on 58 bladder transitional cell carcinoma patients with full clinical follow-up. Median age at diagnosis was 69 years (range 43–91). Median follow-up was 33 months (range 1–180). In all, 27 patients died of their disease.

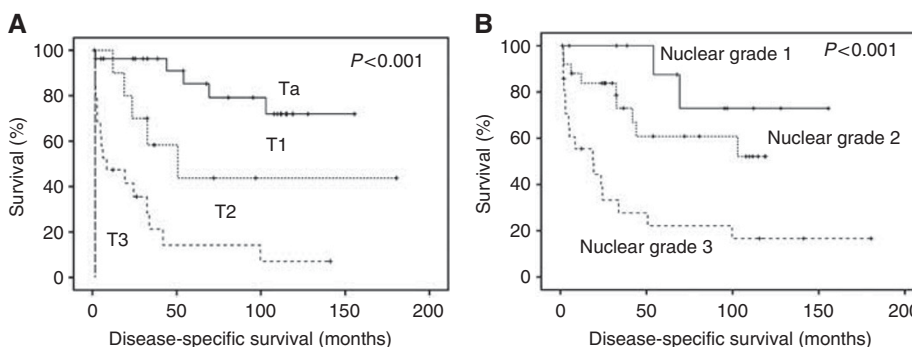
Initial analysis was based on known clinico-pathological features, which are known to be prognostic indicators for survival

in bladder cancer. T-stage (Figure 1A) and tumour grade (Figure 1B) were significantly associated with poor prognosis, thus validating our cohort for use in a biomarker study (Table 1).

Each cellular location was independently assessed for expression levels (Figure 2). Tumours were divided into those with high (above median) or low (below or equal to the median) expression.

**c-Src kinase**

Of the tumours investigated, 74% showed some nuclear expression, 100% cytoplasmic expression, and 93% membrane expression. Tumours were subdivided into with high (above median) and low (below median) expression. The  $\chi^2$ -analysis demonstrated that membrane c-Src negatively correlated with tumour grade (*P*=0.024, Table 2), but no correlation was demonstrated with age, T-stage, recurrence or metastases. These results suggested that when located in the membrane, c-Src conferred a good prognosis; however, on univariate analysis, expression of c-Src at the different cellular locations did not show significance (Table 1), therefore no correlation with cancer-specific survival was observed for this marker. Pearson's correlation demonstrated that membrane c-Src expression positively correlated with cytoplasmic c-Src (*P*<0.0001, Table 3).



**Figure 1** (A) Shows a Kaplan–Meier plotted for T-stage against cancer-specific survival, log rank test *P*<0.001. (B) Shows a Kaplan–Meier plotted grade against cancer-specific survival, log rank test *P*<0.001.

**Table 1** Impact of clinicopathological factors and protein expression/activation on patient survival

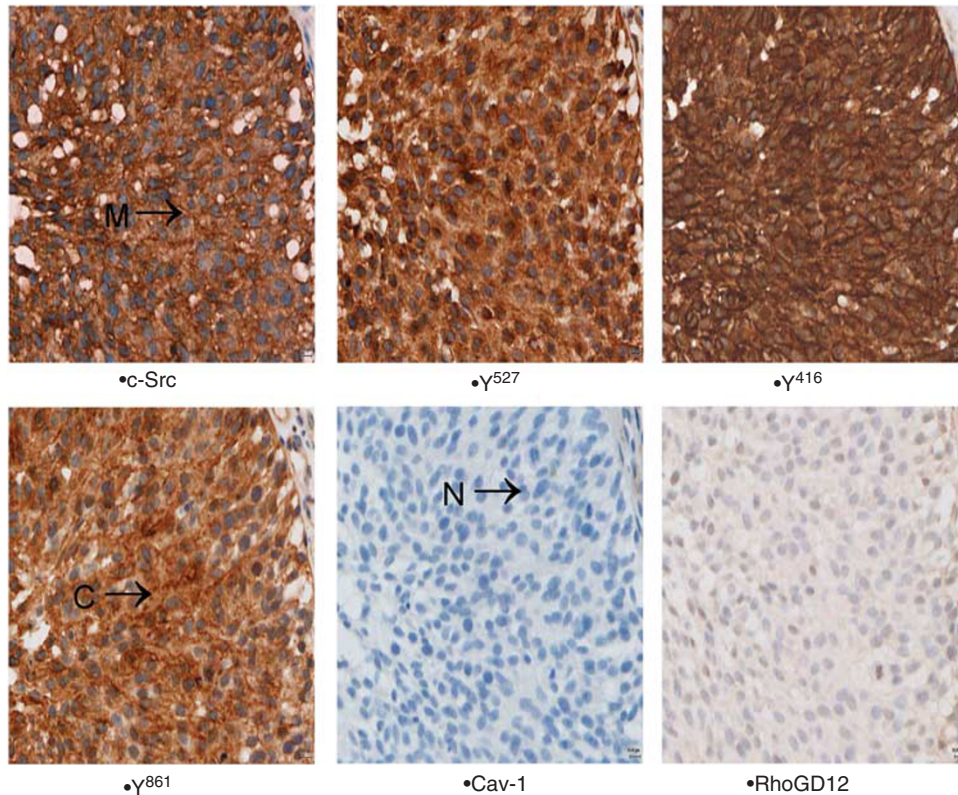
	Numbers	Univariate analysis	Multivariate analysis	HR
		<i>P</i> -value	<i>P</i> -value	
Age (<60/>60)	23/35	0.287		
T-stage (Ta/T1/T2/T3/T4)	27/20/10/1/0	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	3.22 (1.97–5.24)
Grade (1/2/3)	11/25/22	<b>&lt; 0.001</b>	—	—
c-Src nuc (negative/positive)	37/21	0.418	—	—
c-Src cyto (negative/positive)	31/27	0.308	—	—
c-Src mem (negative/positive)	30/28	0.22	—	—
Y <sup>527</sup> Src nuc (negative/positive)	56/2	<b>0.008</b>	—	—
Y <sup>527</sup> Src cyto (negative/positive)	37/21	0.405	—	—
Y <sup>527</sup> Src mem (negative/positive)	26/32	<b>0.01</b>	—	—
Y <sup>416</sup> Src nuc (negative/positive)	30/28	0.647	—	—
Y <sup>416</sup> Src cyto (negative/positive)	29/29	0.264	—	—
Y <sup>416</sup> Src mem (negative/positive)	33/25	<b>0.001</b>	<b>0.008</b>	0.288 (0.11–0.72)
Y <sup>861</sup> FAK nuc (negative/positive)	52/6	0.995	—	—
Y <sup>861</sup> FAK cyto (negative/positive)	38/20	0.732	—	—
Y <sup>861</sup> FAK mem (negative/positive)	25/33	<b>0.008</b>	—	—
Cav nuc (negative/positive)	57/1	<b>0.042</b>	—	—
Cav cyto (negative/positive)	31/27	0.689	—	—
Cav mem (negative/positive)	44/14	0.177	—	—
Rho nuc (negative/positive)	29/29	0.186	—	—
Rho cyto (negative/positive)	30/28	0.712	—	—
Rho mem (negative/positive)	31/27	0.425	—	—

Abbreviations: Cav = caveolin; cyto = cytoplasmic; FAK = focal adhesion kinase; HR = hazards ratio; mem = membrane; nuc = nuclear. Each clinical and pathological parameter was correlated to survival (*P*-values). Figures in bold denote significant values.

### Y<sup>527</sup> Src kinase

Of the tumours investigated, 3% showed some degree of nuclear expression, 98% cytoplasmic expression, and 93% membrane expression. The  $\chi^2$ -analysis demonstrated that expression of membrane-dephosphorylated Y<sup>527</sup> negatively correlated with T-stage and grade ( $P=0.021$ ,  $P=0.011$ , Table 2), but no correlation was demonstrated with age, recurrence or metastases. On univariate analysis, high expression of membrane-dephosphorylated Y<sup>527</sup> was associated with increased cancer-specific

survival ( $P=0.01$ , Table 1). In contrast, high expression of nuclear-dephosphorylated Y<sup>527</sup> was associated with decreased cancer-specific survival,  $P=0.008$ ; however, this result should be viewed with caution, as only 3% of tumours exhibited high nuclear-dephosphorylated Y<sup>527</sup> expression, (Table 1). Pearson's correlation demonstrated expression of membrane Y<sup>527</sup> positively correlated with membrane c-Src expression ( $P<0.0001$ , Table 3), suggesting that both surrogate markers of c-Src activation correlate with each other, and therefore validating their use in this manner.



**Figure 2** Shows representative images of immunohistochemistry for bladder cancer of Src kinase, Src Y<sup>527</sup>, Src Y<sup>416</sup> and FAK Y<sup>861</sup>. Membrane staining denoted by M, cytoplasmic staining by C and nuclear staining N.

**Table 2** Interrelationships between clinicopathological characteristics of patients and protein expression/activation with bladder cancer using  $\chi^2$ -analysis

Variable	Numbers	Age	T-stage	Grade	Recurrence	Metastases at follow-up
c-Src nuc (negative/positive)	37/21	0.139	0.814	0.706	0.75	0.159
c-Src cyto (negative/positive)	31/27	0.49	0.515	0.689	0.563	0.563
c-Src mem (negative/positive)	30/28	0.956	0.066	<b>0.024*</b>	0.448	0.416
Y <sup>527</sup> Src nuc (negative/positive)	56/2	0.763	0.097	0.544	0.198	0.113
Y <sup>527</sup> Src cyto (negative/positive)	37/21	0.197	0.269	0.706	0.822	0.388
Y <sup>527</sup> Src mem (negative/positive)	26/32	0.483	<b>0.021*</b>	<b>0.011*</b>	0.162	0.078
Y <sup>416</sup> Src nuc (negative/positive)	30/28	0.557	0.693	0.094	0.416	0.773
Y <sup>416</sup> Src cyto (negative/positive)	29/29	0.79	0.21	0.109	0.192	0.295
Y <sup>416</sup> Src mem (negative/positive)	33/25	0.304	0.054	<b>0.005*</b>	0.14	<b>0.001*</b>
Y <sup>861</sup> FAK nuc (negative/positive)	52/6	0.588	0.825	0.211	0.553	0.79
Y <sup>861</sup> FAK cyto (negative/positive)	38/20	0.602	0.936	0.295	0.569	0.595
Y <sup>861</sup> FAK mem (negative/positive)	25/33	0.963	0.083	0.125	<b>0.026*</b>	<b>0.045*</b>
Cav nuc (negative/positive)	57/1	0.418	0.245	0.267	0.367	0.267
Cav cyto (negative/positive)	31/27	0.49	0.352	0.689	0.638	0.957
Cav mem (negative/positive)	44/14	0.335	0.09	<b>0.026</b>	0.658	0.292
Rho nuc (negative/positive)	29/29	0.425	0.21	0.593	0.601	<b>0.036*</b>
Rho cyto (negative/positive)	30/28	0.956	0.693	0.547	0.814	0.814
Rho mem (negative/positive)	31/27	0.363	0.851	0.689	0.32	0.957

Abbreviations: Cav = caveolin; cyto = cytoplasmic; FAK = focal adhesion kinase; mem = membrane; nuc = nuclear. Figures in bold denote significant values. \*Negative correlation.

**Table 3** Interrelationships between protein markers at the various cellular locations using Pearson's correlation

	c-Src nuc	c-Src cyto	c-Src mem	Y <sup>27</sup> Src nuc	Y <sup>27</sup> Src cyto	Y <sup>27</sup> Src mem	Y <sup>16</sup> Src nuc	Y <sup>16</sup> Src cyto	Y <sup>16</sup> Src mem	Y <sup>861</sup> FAK nuc	Y <sup>861</sup> FAK cyto	Y <sup>861</sup> FAK mem	Cav nuc	Cav cyto	Cav mem	Rho nuc	Rho cyto	Rho mem	
c-Src nuc	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
c-Src cyto	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
c-Src mem	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>27</sup> Src nuc	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>27</sup> Src cyto	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>27</sup> Src mem	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>16</sup> Src nuc	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>16</sup> Src cyto	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>16</sup> Src mem	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>861</sup> FAK nuc	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>861</sup> FAK cyto	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y <sup>861</sup> FAK mem	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cav nuc	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cav cyto	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PC	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig	—	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 3 (Continued)

	c-Src nuc	c-Src cyto	c-Src mem	Y <sup>527</sup> Src nuc	Y <sup>527</sup> Src cyto	Y <sup>527</sup> Src mem	Y <sup>416</sup> Src nuc	Y <sup>416</sup> Src cyto	Y <sup>416</sup> Src mem	Y <sup>861</sup> FAK nuc	Y <sup>861</sup> FAK cyto	Y <sup>861</sup> FAK mem	Cav nuc	Cav cyto	Cav mem	Rho nuc	Rho cyto	Rho mem
Cav mem	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	NS	NS	NS
Sig	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	NS	NS	NS
Rho nuc	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.6326	NS
PC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<b>P &lt; 0.0001</b>	NS
Sig	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<b>P &lt; 0.0001</b>	NS
Rho cyto	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.5600
PC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<b>P &lt; 0.0001</b>
Sig	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<b>P &lt; 0.0001</b>
Rho mem	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sig	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations: Cav = caveolin; cyto = cytoplasmic; mem = membrane; nuc = nuclear; NS = non-significant P-values; PC = Pearson's correlations; Sig = significance (2-tailed). Figures in bold denote significant values.

Y<sup>416</sup> Src kinase

Of the tumours investigated, 90% showed some degree of nuclear expression, 100% cytoplasmic expression, and 100% membrane expression. The  $\chi^2$ -analysis demonstrated that expression of membrane Y<sup>416</sup> negatively correlated with tumour grade and evidence of metastasis at follow-up ( $P=0.005$ ,  $P=0.001$ , Table 2), but no correlation was demonstrated with age, T-stage and recurrence. On univariate analysis, high expression of membrane Y<sup>416</sup> was associated with increased cancer-specific survival ( $P=0.001$ , Table 1, Figure 3A) and was shown to be an independent factor on multivariate analysis ( $P=0.008$ , HR 0.288, CI 0.11–0.72, Table 1).

FAK Y<sup>861</sup>

Of the tumours investigated, 10% showed some degree of nuclear expression, 34% cytoplasmic expression, and 91% membrane expression. The  $\chi^2$ -analysis demonstrated that expression of membrane Y<sup>861</sup> negatively correlated with recurrence and evidence of metastases ( $P=0.026$ ,  $P=0.045$ , Table 2), but no correlation was demonstrated with age, T-stage or grade. On univariate analysis, high expression of membrane Y<sup>861</sup> was associated with increased cancer-specific survival ( $P=0.008$ , Table 1, Figure 3B). Pearson's correlation demonstrated the expression of cytoplasmic FAK Y<sup>861</sup> demonstrated a positive correlation with cytoplasmic Y<sup>527</sup> ( $P<0.0001$ , Table 3). Expression of membrane FAK Y<sup>861</sup> demonstrated a positive correlation with membrane c-Src, Y<sup>527</sup> and Y<sup>416</sup> ( $P<0.0001$ ,  $P<0.0001$ ,  $P=0.0001$ , Table 3). Therefore, all markers of Src activation correlated with activation of the downstream marker FAK Y<sup>861</sup>.

Caveolin-1

Of the tumours investigated, 2% showed degree of nuclear expression, 97% cytoplasmic expression, and 24% membrane expression. The  $\chi^2$ -analysis demonstrated that expression of membrane Cav-1 positively correlated with tumour grade ( $P=0.026$ , Table 2), but no correlation was demonstrated with age, T-stage, recurrence or metastases. On univariate analysis, however, high expression of nuclear Cav-1 was associated with decreased cancer-specific survival ( $P=0.042$ , Table 1), but as only 2% of tumours exhibited high nuclear expression, these results need to be viewed with caution.

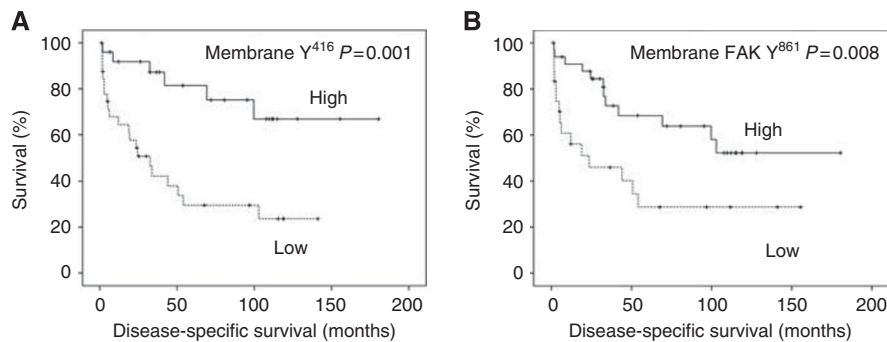
RhoGD12

Of the tumours investigated, 91% showed degree of nuclear expression, 91% cytoplasmic expression, and 71% membrane expression. The  $\chi^2$ -analysis demonstrated that expression of nuclear RhoGD12 had a negative correlation with metastases ( $P=0.036$ , Table 2), but no correlation was demonstrated with age, T-stage, grade or recurrence. Pearson's correlation demonstrated expression of cytoplasmic RhoGD12 demonstrated a positive correlation with nuclear RhoGD12 ( $P<0.0001$ , Table 3). Expression of membrane RhoGD12 demonstrated a positive correlation with cytoplasmic and membrane c-Src ( $P<0.0001$ ,  $P<0.0001$ , Table 3) and cytoplasmic RhoGD12 expression ( $P<0.0001$ , Table 3). This suggests that RhoGD12 expression is associated with levels of c-Src in bladder cancer.

DISCUSSION

To our knowledge, this is the only study investigating the role of Src kinase, dephosphorylation status (Y<sup>530</sup>), autophosphorylation status (Y<sup>419</sup>), the downstream signalling protein FAK Y<sup>861</sup>, Cav-1 and RhoGD12 expression in one cohort of bladder cancers.

This study demonstrates that when c-Src is active (utilising membrane expression as a surrogate marker of activation), an



**Figure 3** (A) Shows a Kaplan–Meier plotted for high and low membrane  $Y^{416}$  expression against cancer-specific survival, log rank test  $P=0.001$ . (B) Shows a Kaplan–Meier plotted for high and low membrane FAK  $Y^{861}$  expression against cancer-specific survival, log rank test  $P=0.008$ .

inverse correlation with tumour grade is observed. These results are consistent with those that have previously reported a negative correlation of Src expression with bladder aggressiveness (Fanning *et al*, 1992; Blaveri *et al*, 2005; Sanchez-Carbayo *et al*, 2006; Wu *et al*, 2009; Thomas *et al*, 2011), and further reiterates that Src inhibitors should be utilised with much caution for cancer prevention in transitional cell carcinoma.

It has been suggested that a biomarker for prediction of Src kinase activity would be to measure phosphorylation of the protein at a site associated with activity (Bolen *et al*, 1987; Masaki *et al*, 1998), and Src kinase, when active, translocates to the membrane (Frame, 2002). When examining the expression of various phosphorylated statuses of Src, we demonstrated that expression of dephosphorylated Src (dephosphorylated  $Y^{527}$ ) and autophosphorylated Src ( $Y^{416}$ ) negatively correlated with tumour grade. Furthermore, increased expression of membrane Src  $Y^{416}$  was an independent predictor of improved cancer-specific survival. The antibody for  $Y^{419}$  is not specific for one particular Src family member, but because of sequence homogeneity, cross reacts with all Src family members that are phosphorylated at this site. Therefore, as c-Src itself in this study is not associated with clinical outcome measures, we hypothesise that expression of another of the SFK members is driving the association with improved outcome observed in the current study. Further evidence supporting the observation that activation of Src family members confers an improved prognosis is by assessing the relationships of the downstream signalling protein FAK  $Y^{861}$  to outcome. Expression of FAK  $Y^{861}$  was observed to be negatively associated with recurrence, presence of metastases and increased cancer-specific survival.

Cav-1 expression can have both a positive and negative effect on cancer progression. In this study, when assessing expression of Cav-1, it was shown that presence in the membrane positively correlated with nuclear grade. This is also in keeping with previous work which has shown that Cav-1 expression was associated with grade (Rajjayabun *et al*, 2001; Fong *et al*, 2003; Kunze *et al*, 2006). It has also been demonstrated that Cav-1 expression was more membranous in those with ovarian cancer and had short-term survival (Fine *et al*, 2001), which is a similar finding to what we have shown that membranous expression has a positive correlation with grade, and this factor is associated with poorer survival. Previous work has shown that Cav-1 and Src have a reciprocal relationship in bladder cancer (Thomas *et al*, 2011). In this study, no such relationship reached significance, but it was demonstrated

that Cav-1 and c-Src had a positive correlation and Cav-1 had a negative correlation with  $Y^{416}$ . This also suggests that it is another of the SFK member, which is associated with a reciprocal relationship with Cav-1. It has been suggested that Cav-1 inhibits Src through recruitment of the C-terminal (Li *et al*, 1996; Cao *et al*, 2002), but in this study, there was no correlation between Cav-1 and  $Y^{527}$ .

RhoGD12 expression has been thought of as being a suppressor of cancer progression and metastases, but has also been shown to positively correlate with cancer progression in various malignancies (Tapper *et al*, 2001; Zhang and Zhang, 2006; Cho *et al*, 2009). We have observed that nuclear expression of RhoGD12 negatively correlated with the presence of metastases at follow-up, which is consistent with work showing that RhoGD12 is a metastases suppressor in bladder cancer (Gildea *et al*, 2002; Theodorescu *et al*, 2004). Previous work has reported that expression of Src and RhoGD12 confers improved prognosis in bladder cancer (Fanning *et al*, 1992; Theodorescu *et al*, 2004; Blaveri *et al*, 2005; Sanchez-Carbayo *et al*, 2006; Thomas *et al*, 2011). It has also been reported active Src ( $Y^{530}$ ) results in elevated levels of RhoGD12 (Wu *et al*, 2009). Given that, when Src is dephosphorylated at  $Y^{530}$ , it is not active yet, and this would suggest a relationship between inactive Src and RhoGD12. This study does demonstrate that expression of RhoGD12 is associated with less likelihood of metastases, but also shows a positive relationship between RhoGD12 and membrane c-Src. As c-Src is active when located in the membrane, we have therefore shown that it is active c-Src that has a relationship with RhoGD12.

This current study further reinforces that expression of Cav-1 confers poor prognosis. This study reports that RhoGD12 confers improved prognosis, but has a positive correlation with active c-Src. It has also been demonstrated that expression of dephosphorylated Src ( $Y^{527}$ ), autophosphorylated Src ( $Y^{416}$ ) and the downstream marker FAK  $Y^{861}$  confer improved cancer-specific survival. Furthermore, expression of Src  $Y^{416}$  is an independent factor associated with increased cancer-specific survival, suggesting that expression of another SFK member other than Src itself confers improved prognosis.

## ACKNOWLEDGEMENTS

This work was supported by Think Pink.

## REFERENCES

Advanced Bladder Cancer (ABC) Meta-analysis Collaboration (2005) Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur Urol* 48(2): 202–205

Blaveri E, Simko JP, Korkola JE, Brewer JL, Baehner F, Mehta K, Devries S, Koppie T, Pejavar S, Carroll P, Waldman FM (2005) Bladder cancer outcome and subtype classification by gene expression. *Clin Cancer Res* 11(11): 4044–4055

- Bolen JB, Veillette A, Schwartz AM, DeSeau V, Rosen N (1987) Activation of pp60c-src protein kinase activity in human colon carcinoma. *Proc Natl Acad Sci USA* **84**(8): 2251–2255
- Calalb MB, Polte TR, Hanks SK (1995) Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. *Mol Cell Biol* **15**(2): 954–963
- Calalb MB, Zhang X, Polte TR, Hanks SK (1996) Focal adhesion kinase tyrosine-861 is a major site of phosphorylation by Src. *Biochem Biophys Res Commun* **228**(3): 662–668
- Campbell EJ, McDuff E, Tatarov O, Tovey S, Brunton V, Cooke TG, Edwards J (2008) Phosphorylated c-Src in the nucleus is associated with improved patient outcome in ER-positive breast cancer. *Br J Cancer* **99**(11): 1769–1774
- Cao H, Courchesne WE, Mastick CC (2002) A phosphotyrosine-dependent protein interaction screen reveals a role for phosphorylation of caveolin-1 on tyrosine 14: recruitment of C-terminal Src kinase. *J Biol Chem* **277**(11): 8771–8774
- Cho HJ, Baek KE, Park SM, Kim IK, Choi YL, Cho HJ, Nam IK, Hwang EM, Park JY, Han JY, Kang SS, Kim DC, Lee WS, Lee MN, Oh GT, Kim JW, Lee CW, Yoo J (2009) RhoGDI2 expression is associated with tumor growth and malignant progression of gastric cancer. *Clin Cancer Res* **15**(8): 2612–2619
- Cooper JA, Howell B (1993) The when and how of Src regulation. *Cell* **73**(6): 1051–1054
- Dalbagni G, Genega E, Hashibe M, Zhang ZF, Russo P, Herr H, Reuter V (2001) Cystectomy for bladder cancer: a contemporary series. *J Urol* **165**(4): 1111–1116
- Davidson B, Nesland JM, Goldberg I, Kopolovic J, Gotlieb WH, Bryne M, Ben-Baruch G, Berner A, Reich R (2001) Caveolin-1 expression in advanced-stage ovarian carcinoma – a clinicopathologic study. *Gynecol Oncol* **81**(2): 166–171
- Elsberger B, Tan BA, Mitchell TJ, Brown SB, Mallon EA, Tovey SM, Cooke TG, Brunton VG, Edwards J (2009) Is expression or activation of Src kinase associated with cancer-specific survival in ER-, PR- and HER2-negative breast cancer patients? *Am J Pathol* **175**(4): 1389–1397
- Engen JR, Wales TE, Hochrein JM, Meyn III MA, Banu OS, Bahar I, Smithgall TE (2008) Structure and dynamic regulation of Src-family kinases. *Cell Mol Life Sci* **65**(19): 3058–3073
- Epstein JI, Amin MB, Reuter VR, Mostofi FK (1998) The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. *Am J Surg Pathol* **22**(12): 1435–1448
- Fanning P, Bulovas K, Saini KS, Libertino JA, Joyce AD, Summerhayes IC (1992) Elevated expression of pp60c-src in low grade human bladder carcinoma. *Cancer Res* **52**(6): 1457–1462
- Fine SW, Lisanti MP, Galbiati F, Li M (2001) Elevated expression of caveolin-1 in adenocarcinoma of the colon. *Am J Clin Pathol* **115**(5): 719–724
- Fong A, Garcia E, Gwynn L, Lisanti MP, Fazzari MJ, Li M (2003) Expression of caveolin-1 and caveolin-2 in urothelial carcinoma of the urinary bladder correlates with tumor grade and squamous differentiation. *Am J Clin Pathol* **120**(1): 93–100
- Frame MC (2002) Src in cancer: deregulation and consequences for cell behaviour. *Biochim Biophys Acta* **1602**(2): 114–130
- Galbiati F, Volonte D, Engelman JA, Watanabe G, Burk R, Pestell RG, Lisanti MP (1998) Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. *EMBO J* **17**(22): 6633–6648
- Galbiati F, Volonte D, Liu J, Capozza F, Frank PG, Zhu L, Pestell RG, Lisanti MP (2001) Caveolin-1 expression negatively regulates cell cycle progression by inducing G(0)/G(1) arrest via a p53/p21(WAF1/Cip1)-dependent mechanism. *Mol Biol Cell* **12**(8): 2229–2244
- Gildea JJ, Seraj MJ, Oxford G, Harding MA, Hampton GM, Moskaluk CA, Frierson HF, Conaway MR, Theodorescu D (2002) RhoGDI2 is an invasion and metastasis suppressor gene in human cancer. *Cancer Res* **62**(22): 6418–6423
- Glenney Jr JR, Zokas L (1989) Novel tyrosine kinase substrates from Rous sarcoma virus-transformed cells are present in the membrane skeleton. *J Cell Biol* **108**(6): 2401–2408
- Grande-Garcia A, Echarri A, de RJ, Alderson NB, Waterman-Storer CM, Valdivielso JM, del Pozo MA (2007) Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. *J Cell Biol* **177**(4): 683–694
- Ho CC, Huang PH, Huang HY, Chen YH, Yang PC, Hsu SM (2002) Up-regulated caveolin-1 accentuates the metastasis capability of lung adenocarcinoma by inducing filopodia formation. *Am J Pathol* **161**(5): 1647–1656
- Ito Y, Yoshida H, Nakano K, Kobayashi K, Yokozawa T, Hirai K, Matsuzuka F, Matsuura N, Kakudo K, Kuma K, Miyauchi A (2002) Caveolin-1 overexpression is an early event in the progression of papillary carcinoma of the thyroid. *Br J Cancer* **86**(6): 912–916
- Kirkegaard T, Edwards J, Tovey S, McGlynn LM, Krishna SN, Mukherjee R, Tam L, Munro AF, Dunne B, Bartlett JM (2006) Observer variation in immunohistochemical analysis of protein expression, time for a change? *Histopathology* **48**(7): 787–794
- Kopetz S, Shah AN, Gallick GE (2007) Src continues aging: current and future clinical directions. *Clin Cancer Res* **13**(24): 7232–7236
- Kunze E, Von BF, Werner C, Wendt M, Schlott T (2006) Transitional cell carcinomas and nonurothelial carcinomas of the urinary bladder differ in the promoter methylation status of the caveolin-1, hDAB2IP and p53 genes, but not in the global methylation of Alu elements. *Int J Mol Med* **17**(1): 3–13
- Lee H, Volonte D, Galbiati F, Iyengar P, Lublin DM, Bregman DB, Wilson MT, Campos-Gonzalez R, Bouzahzah B, Pestell RG, Scherer PE, Lisanti MP (2000) Constitutive and growth factor-regulated phosphorylation of caveolin-1 occurs at the same site (Tyr-14) *in vivo*: identification of a c-Src/Cav-1/Grb7 signaling cassette. *Mol Endocrinol* **14**(11): 1750–1775
- Li S, Couet J, Lisanti MP (1996) Src tyrosine kinases, Galpha subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. *J Biol Chem* **271**(46): 29182–29190
- Liu P, Rudick M, Anderson RG (2002) Multiple functions of caveolin-1. *J Biol Chem* **277**(44): 41295–41298
- Luo FR, Barrett YC, Yang Z, Camuso A, McGlinchey K, Wen ML, Smykla R, Fager K, Wild R, Palme H, Galbraith S, Blackwood-Chirchir A, Lee FY (2008) Identification and validation of phospho-SRC, a novel and potential pharmacodynamic biomarker for dasatinib (SPRYCEL), a multi-targeted kinase inhibitor. *Cancer Chemother Pharmacol* **62**(6): 1065–1074
- Masaki T, Okada M, Shiratori Y, Rengifo W, Matsumoto K, Maeda S, Kato N, Kanai F, Komatsu Y, Nishioka M, Omata M (1998) pp60c-src activation in hepatocellular carcinoma of humans and LEC rats. *Hepatology* **27**(5): 1257–1264
- Moon HG, Jeong SH, Ju YT, Jeong CY, Lee JS, Lee YJ, Hong SC, Choi SK, Ha WS, Park ST, Jung EJ (2010) Up-regulation of RhoGDI2 in human breast cancer and its prognostic implications. *Cancer Res Treat* **42**(3): 151–156
- Nam S, Kim D, Cheng JQ, Zhang S, Lee JH, Buettner R, Mirosevich J, Lee FY, Jove R (2005) Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res* **65**(20): 9185–9189
- Niu H, Li H, Xu C, He P (2010) Expression profile of RhoGDI2 in lung cancers and role of RhoGDI2 in lung cancer metastasis. *Oncol Rep* **24**(2): 465–471
- Rajjayabun PH, Garg S, Durkan GC, Charlton R, Robinson MC, Mellon JK (2001) Caveolin-1 expression is associated with high-grade bladder cancer. *Urology* **58**(5): 811–814
- Sanchez-Carbayo M, Socci ND, Lozano J, Saint F, Cordon-Cardo C (2006) Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. *J Clin Oncol* **24**(5): 778–789
- Schaller MD, Hildebrand JD, Shannon JD, Fox JW, Vines RR, Parsons JT (1994) Auto-phosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. *Mol Cell Biol* **14**(3): 1680–1688
- Slack NH, Bross ID, Prout Jr GR (1977) Five-year follow-up results of a collaborative study of therapies for carcinoma of the bladder. *J Surg Oncol* **9**(4): 393–405
- Smith Jr JA, Crawford ED, Paradelo JC, Blumenstein B, Herschman BR, Grossman HB, Christie DW (1997) Treatment of advanced bladder cancer with combined preoperative irradiation and radical cystectomy vs radical cystectomy alone: a phase III intergroup study. *J Urol* **157**(3): 805–807
- Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, Skinner E, Bochner B, Thangathurai D, Mikhail M, Raghavan D, Skinner DG (2001) Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. *J Clin Oncol* **19**(3): 666–675



- Stein JP, Skinner DG (2006) Radical cystectomy for invasive bladder cancer: long-term results of a standard procedure. *World J Urol* **24**(3): 296–304
- Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffieux C, Denis L, Newling DW, Kurth K (2006) Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. *Eur Urol* **49**(3): 466–465
- Tapper J, Kettunen E, El-Rifai W, Seppala M, Andersson LC, Knuutila S (2001) Changes in gene expression during progression of ovarian carcinoma. *Cancer Genet Cytogenet* **128**(1): 1–6
- Tatarov O, Mitchell TJ, Seywright M, Leung HY, Brunton VG, Edwards J (2009) SRC family kinase activity is up-regulated in hormone-refractory prostate cancer. *Clin Cancer Res* **15**(10): 3540–3549
- Theodorescu D, Sapinoso LM, Conaway MR, Oxford G, Hampton GM, Frierson Jr HF (2004) Reduced expression of metastasis suppressor RhoGDI2 is associated with decreased survival for patients with bladder cancer. *Clin Cancer Res* **10**(11): 3800–3806
- Thomas S, Overdevest JB, Nitz MD, Williams PD, Owens CR, Sanchez-Carbayo M, Frierson HF, Schwartz MA, Theodorescu D (2011) Src and caveolin-1 reciprocally regulate metastasis via a common downstream signaling pathway in bladder cancer. *Cancer Res* **71**(3): 832–841
- Volonte D, Zhang K, Lisanti MP, Galbiati F (2002) Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. *Mol Biol Cell* **13**(7): 2502–2517
- Wu Y, Moissoglu K, Wang H, Wang X, Frierson HF, Schwartz MA, Theodorescu D (2009) Src phosphorylation of RhoGDI2 regulates its metastasis suppressor function. *Proc Natl Acad Sci USA* **106**(14): 5807–5812
- Zhang Y, Zhang B (2006) D4-GDI, a Rho GTPase regulator, promotes breast cancer cell invasiveness. *Cancer Res* **66**(11): 5592–5598

This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License.