Expression of steroid receptor coactivator 3 in ovarian epithelial cancer is a poor prognostic factor and a marker for platinum resistance

FULL PAPER

BJC

British Journal of Cancer (2013) 108, 2039–2044 | doi: 10.1038/bjc.2013.199

Keywords: ovarian cancer; SRC3; platinum; resistance

Expression of steroid receptor coactivator 3 in ovarian epithelial cancer is a poor prognostic factor and a marker for platinum resistance

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Background: Steroid receptor coactivator 3 (SRC3) is an important coactivator of a number of transcription factors and is associated with a poor outcome in numerous tumours. Steroid receptor coactivator 3 is amplified in 25% of epithelial ovarian cancers (EOCs) and its expression is higher in EOCs compared with non-malignant tissue. No data is currently available with regard to the expression of SRC-3 in EOC and its influence on outcome or the efficacy of treatment.

Methods: Immunohistochemistry was performed for SRC3, oestrogen receptor-α, HER2, PAX2 and PAR6, and protein expression was quantified using automated quantitative immunofluorescence (AQUA) in 471 EOCs treated between 1991 and 2006 with cytoreductive surgery followed by first-line treatment platinum-based therapy, with or without a taxane.

Results: Steroid receptor coactivator 3 expression was significantly associated with advanced stage and was an independent prognostic marker. High expression of SRC3 identified patients who have a significantly poorer survival with single-agent carboplatin chemotherapy, while with carboplatin/paclitaxel treatment such a difference was not seen.

Conclusion: Steroid receptor coactivator 3 is a poor prognostic factor in EOCs and appears to identify a population of patients who would benefit from the addition of taxanes to their chemotherapy regimen, due to intrinsic resistance to platinum therapy.

Ovarian cancer is the second most common gynaecological cancer but the most lethal, with over 200 000 cases diagnosed and 140 000 deaths worldwide per year (Ferlay *et al*, 2010), and in the majority of cases presents with disease that has spread beyond the pelvis. Surgical debulking and systemic chemotherapy with platinum/taxanes are the mainstays of treatment, and despite treatment advances the 5-year survival remains poor. There is good evidence that platinum–taxane first-line chemotherapy is superior to other chemotherapy regimens for ovarian cancer (Thigpen *et al*, 2011), but 20–30% of patients do not respond to this therapy. Experimental models of ovarian cancer have demonstrated that expression of the oestrogen receptor-α (ERα) is associated with a growth response to oestrogen, and in these models growth inhibition occurs with anti-oestrogen both *in vitro* and *in vivo* (Langdon *et al*, 1990, 1993, 1994a, b). In addition, within this context oestrogen was shown to regulate a number of known ER-regulated genes (Langdon *et al*, 1994a, b, 1998). Studies have subsequently utilised endocrine therapy in the clinical setting in the form of the selective oestrogen receptor modulator tamoxifen or inhibition of aromatase. Response rates of 13–17% have been

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Received 10 October 2012; revised 18 March 2013; accepted 8 April 2013; published online 7 May 2013

reported in ovarian cancer with tamoxifen (Hatch *et al*, 1991; Ahlgren *et al*, 1993; Markman *et al*, 1996), while with aromatase inhibitors radiological response rates of 0–15% and marker response in 9–15% have been documented (Bowman *et al*, 2002; del Carmen *et al*, 2003; Papadimitriou *et al*, 2004). Benefit to treatment has been linked to a higher expression of ER (Bowman *et al*, 2002), and a subsequent study, which selected ovarian cancers based on an ER histoscore of >150, found a higher marker and radiological response rate with letrozole in these cases as compared with previous studies (Smyth *et al*, 2007). Furthermore, similar to breast cancer HER2 is lower in endocrine-responsive ovarian tumours (Bowman *et al*, 2002; Smyth *et al*, 2007).

Co-activators are essential for the transcriptional activation of ligand-bound ER, and one such important cofactor is steroid receptor coactivator 3 (SRC3), a member of the p160 steroid receptor coactivator (SRC) family. Steroid receptor coactivator 3 has been showed to be amplified as well as have elevated expression in malignant tissue as compared with normal tissue (Gojis *et al*, 2010a, b). It also has been shown to correlate with markers of aggressive disease, such as increased Ki-67, larger tumours, lymph node involvement, as well as being associated with a poorer prognosis (Gojis *et al*, 2010a, b) and resistance to endocrine resistance in breast cancer (Gojis *et al*, 2010b). Within the context of breast cancer, chromatin immunoprecipitation-based assays have shown that PARD6B/PAR6 and FER1L3 may be regulated by SRC3 via ER (Labhart *et al*, 2005). In addition, SRC3 can compete with PAX2 for binding to the HER2 *cis*-regulatory element, with a resultant increase in HER2 transcription and cell proliferation (Hurtado *et al*, 2008).

In sporadic ovarian cancer, amplification of SRC3 occurs in 25% of cases, with none seen in familial cases (Tanner *et al*, 2000). Amplification of SRC3 is associated with ER positivity and a poorer overall survival (Tanner *et al*, 2000). In addition, the length of the polyQ region within SRC3 has been associated with time to disease recurrence and overall survival, with a short SRC-3 polyQ genotype (<28 repeats) associated with reduced time to both these events (Li *et al*, 2005). These data suggest a role for SRC3 in the pathogenesis of sporadic ovarian carcinoma and a possible effect on survival.

To date, the expression of SRC3 and its effect on outcome and response to treatment have yet to be explored in ovarian cancer. In this study, the expression of SRC3 in a cohort of ovarian cancers was undertaken and its effect on outcome and response to treatment investigated. In addition, the expression of ERα, HER2, PAX2 and PAR6 were assessed.

PATIENTS AND METHODS

Patients. The study was approved by the Lothian Research Ethics Committee (08/S1101/41). No informed consent (written or verbal) was obtained for use of retrospective tissue samples from the patients within this study, most of whom were deceased, as this was not deemed necessary by the Ethics Committee. The study population consisted of 471 FFPE ovarian tumours treated in the Edinburgh Cancer Centre between 1991 and 2006, as described previously (Faratian *et al*, 2011a, b). Summary patient characteristics are shown in Table 1. Standard treatment included cytoreductive surgery followed by platinum-based therapy, with or without combination with a taxane.

Outcome. Overall survival was calculated from the date of diagnosis (primary surgery) to the date of death by ovarian cancer, or to the date of last follow-up (censored). Patients who died from disease other than ovarian cancer were censored. Tumours were taken from primary site (not metastatic) and before commencement of chemotherapy.

Table 1. Clinicopathologic features of patients and first-line treatment received for ovarian cancer

Characteristic	No.	Percent	Prognostic significance *P*-value
Number of patients	471	100	
Age			0.059
Median age	60.4		
Age range	27–86		
First-line chemotherapy regimen			**0.04**
Platinum-based	283	60.1	
Platinum and taxane	175	37.2	
Other/none	11	2.3	
Unknown	2	0.4	
Stage			**<0.0001**
I	47	10.0	
II	56	11.9	
III	271	57.5	
IV	78	16.6	
Unknown	19	4.0	
Histology			**<0.0001**
Serous	264	56.1	
Clear cell	24	5.1	
Endometrioid	94	20	
Mixed	61	13	
MMMT	0	0	
Mucinous	14	3.0	
Other	12	2.5	

Abbreviation: MMMT = malignant mixed mesodermal tumor

Table 2. Primary antibodies used in this study

Primary antibody	Manufacturer	Catalogue number	Concentration
SRC3	BD Transduction Laboratories	61105	1 : 50
ERα	Neomarkers	RM-9101-S1	1 : 50
HER2	Dako	A0485	1 : 400
PAX2	Abcam	23799	1 : 400
PAR6	Abcam	57838	1 : 100

Abbreviations: ERα = oestrogen receptor-α; SRC3 = steroid receptor coactivator 3.

Immunohistochemistry. Two tissue microarrays (TMAs) containing 0.6-mm cores of tumours were constructed using a previously described methodology (Graham *et al*, 2008). Two tissue microarrays were manually stained in triplicate utilising SRC3, ERα, HER2, PAX2 and PAR6 primary antibodies as detailed in Table 2. All TMA tissue sections were incubated with the primary antibodies for 1 h at room temperature. Protein expression was quantified using AQUA. Immunofluorescence for protein targets was performed using methods described previously (Faratian *et al*, 2011a,b). Pan-cytokeratin antibody was used to identify infiltrating tumour cells, DAPI counterstain to identify nuclei and Cy-5-tyramide detection for target for compartmentalised (tissue and subcellular) analysis of tissue sections.

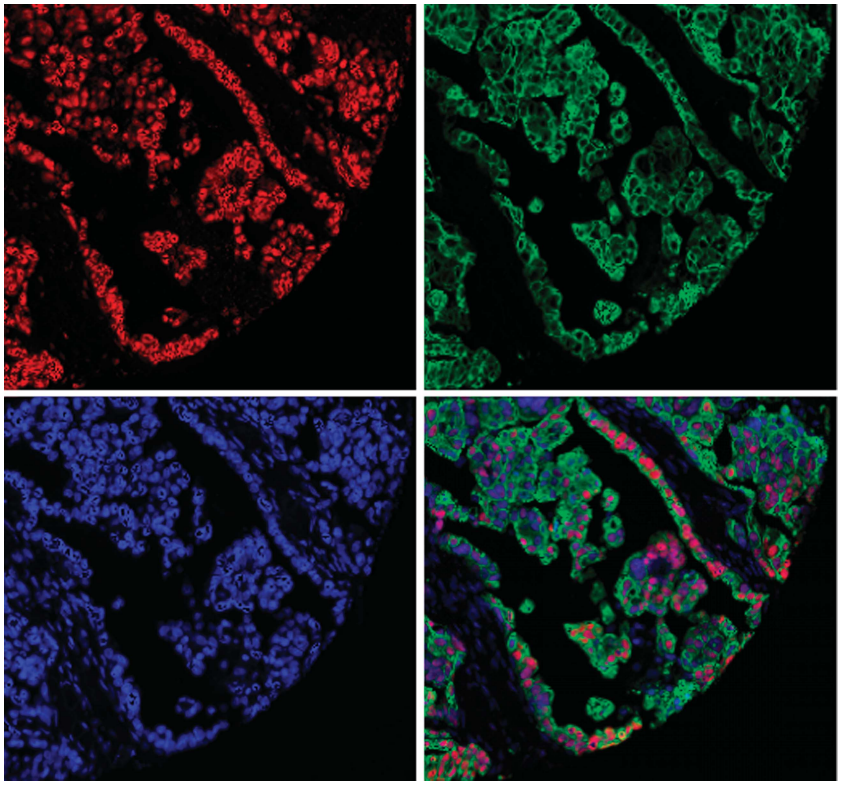


Figure 1. Ovarian tumour core stained for SRC3: red = SRC3; green = cytokeratin; blue = nuclei; combined image = lower right.

Table 3. Correlation between protein concentrations of ER, HER-2, SRC-3, PAX-2 and PAR6

	ERα		HER-2		SRC3		PAX-2		PAR6	
	Correlation coefficient	Significance	Correlation coefficient	Significance	Correlation coefficient	Significance	Correlation coefficient	Significance	Correlation coefficient	Significance
ERα	1.000	—	−0.079	0.09	0.219	<0.001	0.200	<0.001	0.157	<0.001
HER-2	−0.079	0.090	1.000	—	0.190	<0.001	0.172	<0.001	0.159	0.001
SRC3	0.219	<0.001	0.190	<0.001	1.000	—	0.249	<0.001	0.127	0.008
PAX-2	0.200	<0.001	0.172	<0.001	0.249	<0.001	1.000	—	0.382	<0.001
PAR6	0.157	0.001	0.159	<0.001	0.127	0.008	0.382	<0.001	1.000	—

Abbreviations: ERα = oestrogen receptor-α; SRC3 = steroid receptor co-activator 3.

Monochromatic images of each TMA core were captured at ×20 objective using an Olympus AX-51 epifluorescence microscope (Tokyo, Japan), and high-resolution digital images were analysed by the AQUAnalysis software (HistoRx, Branford, CN, USA). If the tumour epithelium comprised <5% of total core area, the core was excluded from analysis, to ensure adequate representation of tissue.

Statistical analyses. Overall survival was assessed by Kaplan–Meier analysis with log-rank testing to determine statistical significance. Univariate and multivariate analyses were performed using Cox proportional hazards regression models. Comparison of differences in means was performed using the Kruskal–Wallis test. To determine the cut-point value for each of the phosphoproteins for Kaplan–Meier analysis, we utilised X-Tile, which allows determination of an optimal cut-point while correcting for the use of minimum *P* statistics, as described previously (Camp *et al*, 2004). Two methods of statistical correction for the use of minimal *P* approach were used, the first calculation of a Monte Carlo *P*-value, and for the second, the Miller–Siegmund minimal *P* correction (Altman *et al*, 1994). All calculations and analyses were two-tailed, where appropriate, and were carried out with SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics. Patient characteristics for the population are summarised in Table 1. The median age for the cohort was 60.4 years (range, 27–86 years); 57.5% (271 out of 471) had stage III

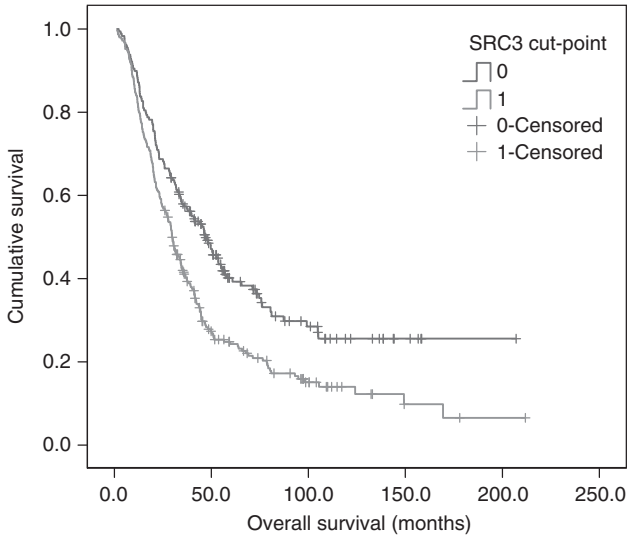
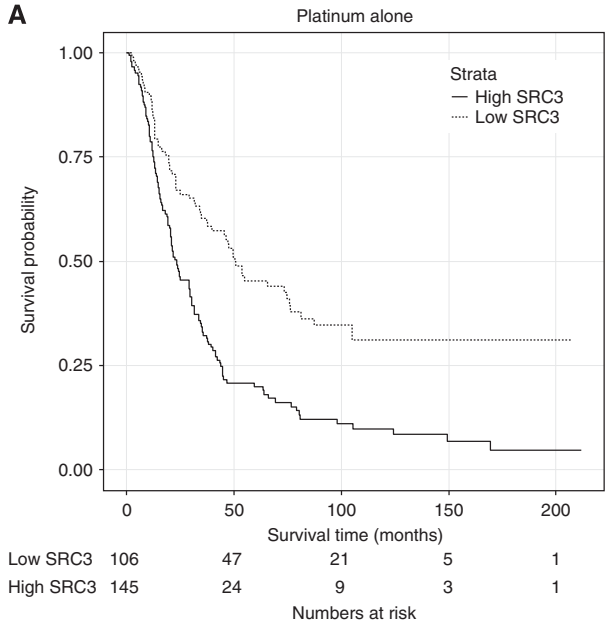


Figure 2. Overall survival based on the expression of SRC3. The colour reproduction of this figure is available on the *British Journal of Cancer* journal online.



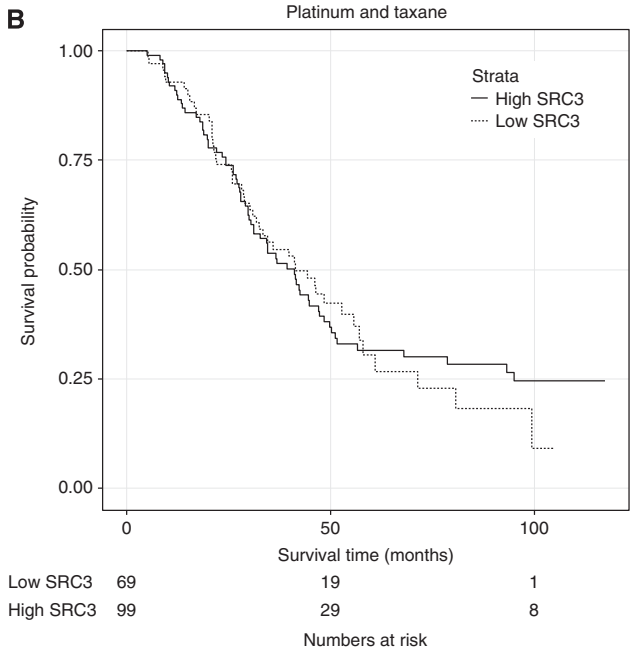


Figure 3. Overall survival based on the expression of SRC3 based on first-line therapy. (**A**) Single-agent platinum treatment and (**B**) platinum and taxane doublet.

tumours and 56% (264 out of 471) had serous type tumours. With regard to first-line treatment, 60% (283 out of 471) received platinum-based treatment, and 37% (175 out of 241) a platinum-taxane doublet.

Correlation of SRC3 with clinicopathological features and other biological parameters. With respect to histopathological parameters, SRC3 expression was significantly higher in stage III and stage IV tumours (Kruskal–Wallis test, $P<0.001$) and lower in endometrioid carcinomas when compared with other histological subtypes (Kruskal–Wallis test, $P<0.001$). Oestrogen receptor was significantly higher in stages III and IV ($P=0.031$), and lower in clear-cell carcinomas (Kruskal–Wallis test, $P<0.0001$); and HER2 was significantly higher in clear-cell and mixed cancers (Kruskal–Wallis test, $P=0.025$). Weak but significant correlations were seen between SRC3 and ERα, HER-2, PAX-2 and PAR6 (Figure 1 and Table 3).

SRC3 and outcome. High expression of SRC3 (as assessed by AQUA) identified patients who have a significantly worse overall survival (Figure 2; *P*-value $\leqslant 0.001$, Miller–Siegmund *P*-value $=0.0029$, Monte-Carlo *P*-value <0.0001). With multivariate analysis, we identified ERα and SRC3 expressions as independent prognostic factors. Stage ($P<0.001$, relative risk $=1.865$), ER expression ($P<0.001$, relative risk $=0.500$), SRC3 expression ($P=0.015$, relative risk $=1.349$) and treatment regimen ($P=0.025$, relative risk $=0.783$).

Expression of SRC3 and outcome of first-line chemotherapy. Expression of SRC3 identified patients who have a significantly improved survival when treated with single-agent carboplatin chemotherapy ($P<0.001$) (Figure 3a), with patients with low SRC3 having a better survival when treated with single carboplatin as compared to those with a high expression. In patients treated with the combination of carboplatin and paclitaxel, this difference is no longer seen in patients with low and high expression having a similar outcome (Figure 3b).

DISCUSSION

This is the first time that data relating to the expression of SRC3 in the context of ovarian cancer and its potential as a prognostic and treatment-predictive marker have been explored. As in other tumour types, high expression of SRC3 was associated with more advanced tumours (Gojis *et al*, 2010a, b), and the significant association with stage of disease is in keeping with the known role of SRC3 in cell motility and invasion (Bai *et al*, 2000; Li *et al*, 2008a, b), which is known to involve focal adhesion turnover and focal adhesion kinase activation (Qin *et al*, 2008), as well as upregulation of the expression of matrix metalloproteinase (Qin *et al*, 2008).

The role of SRC3 as a predictive factor in the response to oncological therapies has been previously explored in the context of endocrine therapy (particularly tamoxifen in breast cancer), but no previous reports have explored its importance in systemic cytotoxic treatments. With regard to tamoxifen and breast cancers, differing results have been reported with reference to SRC3 and its predictive nature. In a retrospective series of breast cancers, high

SRC3 in the presence of tamoxifen was a negative prognostic factor (Osborne *et al*, 2003). However, other retrospective series have found it associated with recurrence on tamoxifen but not with long-term outcome (Dihge *et al*, 2008) or its expression alone had no influence on disease-free survival in tamoxifen-treated patients (Kirkgaard *et al*, 2007). In the context of premenopausal women, who entered into a randomised study of tamoxifen *vs* no tamoxifen, high SRC3 in the presence of tamoxifen treatment was associated with a significantly better disease-free survival (Alkner *et al*, 2010). The reasons for these disparate results are likely to be related to patient heterogeneity as well as methodological issues. In the current cohort, high SRC3 was associated with a significantly poorer overall survival when single-agent carboplatin was utilised as first-line therapy compared to those with low SRC3. In those patients receiving the doublet carboplatin/paclitaxel, there was no difference in outcome based on SRC3 expression. These data would suggest that SRC3 is a potential marker for resistance to single-agent platinum therapy and could be used to identify cases of ovarian cancer that could benefit from carboplatin/paclitaxel combination therapy. The underlying mechanism for the involvement of SRC3 in resistance to single-agent platinum could be via its effect on insulin-like growth factor (IGF) signalling. It has been previously shown that increased IGF-1R mRNA expression is linked with resistance to cisplatin, and IGF-1R mRNA expression has been found to be strongly correlated with intrinsic cisplatin resistance status in a panel of human ovarian cancer cells (Eckstein *et al*, 2009). Steroid receptor coactivator 3 is known to maintain IGF-I in the circulation (Liao *et al*, 2008), and in the context of human breast cancer mediates the effects of IGF-1-induced proliferation, signalling and cell survival (Oh *et al*, 2004). Furthermore, SRC3 is known to be phosphorylated by IGF-1 at tyrosine 1357, which contributes to it oncogenic behaviour (Oh *et al*, 2008). Therefore, it could be hypothesised that the effects of SRC-3 seen in this report are mediated in an IGF-1/IGFR-dependent manner.

A number of large randomised studies have explored the efficacy of paclitaxel in combination with platinum against a platinum-based control treatment as first-line treatment for ovarian cancer. However, only the third International Collaborative Ovarian Neoplasm study (ICON 3) (ICON, 2002) and Gynecology Oncology Group-132 (GOG-132) (Muggia *et al*, 2000) included a randomisation to platinum alone, and in these studies the outcome with paclitaxel/platinum doublet was equivalent to platinum alone. Given the data presented here, it would be of interest to explore the expression of SRC3 and its influence on outcome in cases entered into ICON3 and GOG-132 to confirm its potential usefulness as a potential biomarker for treatment selection.

This study, although it is based on a well-defined and large cohort of 471 patients, which were carefully followed up, is limited by the fact that it is a single-centre retrospective study. Furthermore, given we were unable to explore the potential efficacy of taxane alone. Therefore, these findings need to be explored in the context of ICON 3 (ICON, 2002) and GOG-132 (Muggia *et al*, 2000).

In summary, SRC3 is a poor prognostic factor in ovarian epithelial cancers and appears to identify patients who would benefit from the addition of taxanes to their platinum-based first-line treatment. Further studies of prospective randomised studies are required.

ACKNOWLEDGEMENTS

CP acknowledges a grant support from Cancer Research UK. This work was supported, in part, by the Scottish Funding Council (Grant Number HR07005; http://www.sfc.ac.uk/), Medical Research Scotland and the Cancer Research UK Experimental Centre. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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