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
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 shown 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 ER, 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.

**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, ER, 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 compartmentalised (tissue and subcellular) analysis of tissue sections.

**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                            |

**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.
Monochromatic images of each TMA core were captured at $\times 20$ objective using an Olympus AX-51 epifluorescence microscope (Tokyo, Japan), and high-resolution digital images were analysed by the AQUAAnalysis 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
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 ER\(_a\), 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 \( p < 0.001 \)). With multivariate analysis, we identified ER\(_a\) 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 as a poor prognostic factor and a marker for platinum resistance

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