Tamoxifen-induced ER–α–SRC3 interaction in HER2 positive human breast cancer; a possible mechanism for ER isoform specific recurrence

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The authors and journal apologise for errors in the above paper, which appeared in volume 13, part 4, pages 1135–1145. The original manuscript contains errors in Figure 1C, Figure 2 and Figure 3 (i). The authors would like to apologise for this and have repeated the experiments which recapitulate the findings of the original data. The corrected artwork appears in full here:

(C) SRC3 expression is enhanced in HER2 positive breast cancer patients following endocrine treatment. (i) Table of patient information for endocrine treated patients. SRC3 expression was assessed in primary and metastatic matched tumours from six patients who had failed endocrine treatment (AI, aromatase inhibitor; tam, tamoxifen). Receptor status (ER, PgR and HER2) is described and disease free survival is given in months. (ii) Expression of SRC3 was assessed by immunohistochemistry. Immunohistochemistry was carried using SRC3 (AIB1) antibody (rabbit, sc-25742; Santa Cruz Biotechnology, Santa Cruz, CA, USA) with the Dako EnVision Kit (Dako, Glostrup, Denmark). Expression of SRC3 was found to increase in HER2 positive patients following endocrine treatment but not in HER2 negative patients (representative image shown, n=3 patients/group). (iii) Nuclear staining of SRC3 was assessed using a modified Allred scoring system. SRC3 expression was significantly elevated in metastatic tumours following endocrine treatment in HER2 positive patients (P=0.038, n=3 patients/group, Students t test), but not in HER2 negative patients.

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Tamoxifen induces recruitment of SRC3 to ER target genes in HER2 positive tumour cells. (A) Recruitment of SRC3 to the DNA was assessed using chromatin immunoprecipitation (ChIP). MCF7–HER2 cells were treated with vehicle, estrogen (10^{-8} M), tamoxifen (4-OHT) (10^{-7} M) and/or herceptin (10 ng/ml) for 45 minutes, cross-linked with 1% formaldehyde (F15587; Sigma Aldrich). Immunoprecipitation was performed using a SRC3 antibody (sc-25742) attached to Dynal beads (Dynabeads M-280 Sheep Anti-Rabbit IgG; Life Technologies). The proteins were then removed from the DNA by reverse crosslinking and the DNA was extracted using phenol–chloroform–isoamyl alcohol (P2069; Sigma Aldrich). Real-time PCR was carried out in duplicate by SYBR Green PCR (Qiagen) using a Lightcycler (Roche) and primers for the promoter region of GREB1 and TFF1. Treatment of MCF7–HER2 cells with tamoxifen induced a significant increase in recruitment of SRC3 to the ER target genes GREB1 and TFF1 (P<0.001, Wilcoxon signed rank, n=4). Co-treatment with the HER2 antagonist herceptin inhibits tamoxifen induced SRC3 DNA recruitment. (B) Treatment with tamoxifen does not induce SRC3 recruitment to the ER target GREB1 in parental MCF7 cells. (C) Overexpression of HER2 in MCF7–HER2 cells was confirmed at the transcript level by real time PCR (Lightcycler) (P<0.001, n=3) and at the protein level by western blot (representative blot of three separate experiments).
Tamoxifen induces ER–SRC3 interactions in HER2 positive breast cancer cells. MCF7–HER2 cells were treated with vehicle (V), estrogen (E2) ($10^{-8}$ M) and/or tamoxifen (4-OHT) ($10^{-7}$ M) for 24 h. SRC3 was immunoprecipitated (with anti-SRC3, sc-25742) and the protein was subsequently immunoblotted for ERalpha (mouse, NCL-L-ER-6F11, Leica Biosystems, Nussloch, Germany) or SRC3 (sc-25742). Densitometric analysis of the protein bands provides graphical representation of the relative levels of protein interaction with SRC3.

Figure 3

(i)