

# SCIENTIFIC REPORTS



OPEN

## Recurrent hormone-binding domain truncated *ESR1* amplifications in primary endometrial cancers suggest their implication in hormone independent growth

Received: 24 August 2015

Accepted: 15 April 2016

Published: 10 May 2016

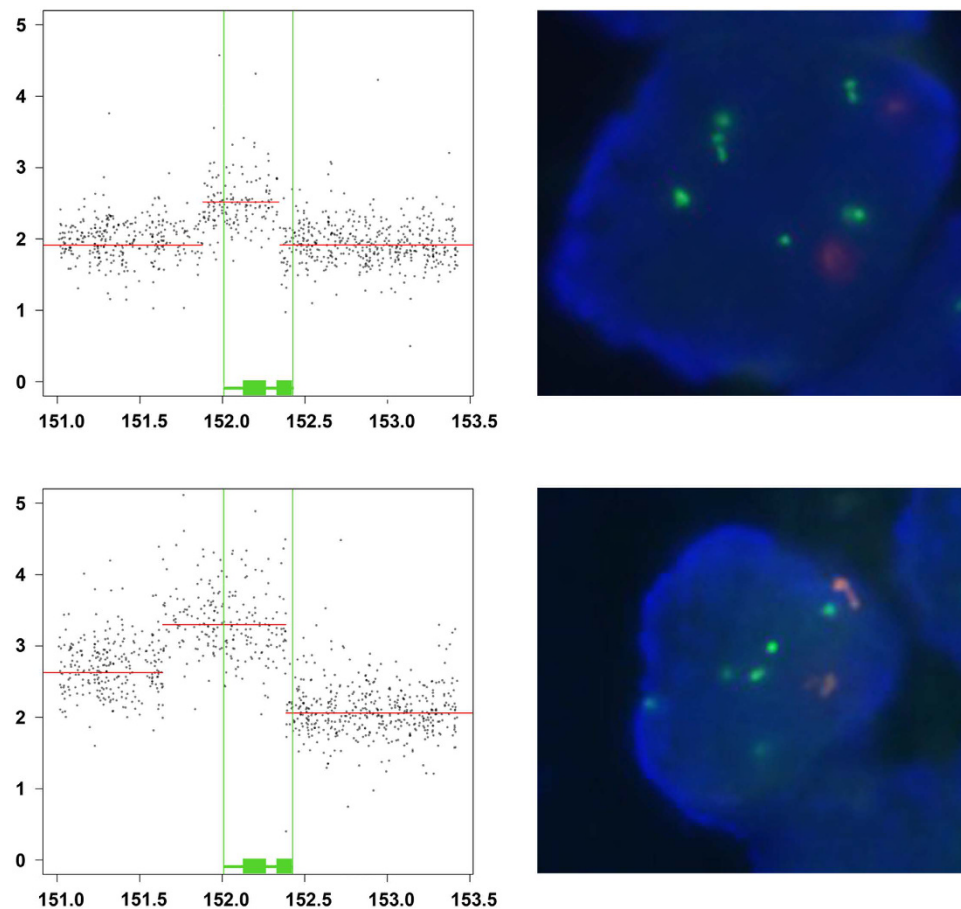
Frederik Holst<sup>1,2,3,4</sup>, Erling A. Hoivik<sup>1,2</sup>, William J. Gibson<sup>3,4,5,6</sup>, Amaro Taylor-Weiner<sup>3,4,5,6</sup>, Steven E. Schumacher<sup>3,4</sup>, Yan W. Asmann<sup>8</sup>, Patrick Grossmann<sup>9,10</sup>, Jone Trovik<sup>1,2</sup>, Brian M. Necela<sup>11</sup>, E. Aubrey Thompson<sup>9</sup>, Matthew Meyerson<sup>4,5,7</sup>, Rameen Beroukhim<sup>3,4,5,6</sup>, Helga B. Salvesen<sup>1,2,†</sup> & Andrew D. Cherniack<sup>4,5</sup>

The estrogen receptor alpha (ER $\alpha$ ) is highly expressed in both endometrial and breast cancers, and represents the most prevalent therapeutic target in breast cancer. However, anti-estrogen therapy has not been shown to be effective in endometrial cancer. Recently it has been shown that hormone-binding domain alterations of ER $\alpha$  in breast cancer contribute to acquired resistance to anti-estrogen therapy. In analyses of genomic data from The Cancer Genome Atlas (TCGA), we observe that endometrial carcinomas manifest recurrent *ESR1* gene amplifications that truncate the hormone-binding domain encoding region of *ESR1* and are associated with reduced mRNA expression of exons encoding the hormone-binding domain. These findings support a role for hormone-binding alterations of ER $\alpha$  in primary endometrial cancer, with potentially important therapeutic implications.

Endometrial cancer (EC) is the fourth most common malignancy of women and the most common pelvic gynecological malignancy in countries with advanced industrialization<sup>1,2</sup>. But approved targeted therapies are still not in use today<sup>3,4</sup>. ER $\alpha$ , encoded by the gene *ESR1*, is known to be an important driver of cell proliferation<sup>5</sup> and has been identified as a risk locus in breast cancer<sup>6,7</sup>. Both breast as well as endometrial cancer are estrogen dependent and express the estrogen receptor alpha (ER $\alpha$ ) to a similar extent<sup>8–11</sup>.

While ER $\alpha$  constitutes the most frequently inhibited therapeutic target in breast cancer<sup>9</sup>, anti-estrogen therapy has shown inconsistent results and mostly a very limited effect in endometrial cancers<sup>12–18</sup>. The estrogen antagonist Tamoxifen can even increase the risk of carcinogenesis<sup>19–21</sup>. Consequently anti-estrogen therapy does not constitute a component of standard therapy of EC<sup>3,4</sup>. Since mutations and alternative splicing of *ESR1*

<sup>1</sup>Centre for Cancer Biomarkers, Department of Clinical Science, The University of Bergen, Norway. <sup>2</sup>Department of Gynecology and Obstetrics, Haukeland University Hospital Bergen, Norway. <sup>3</sup>Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. <sup>4</sup>Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. <sup>5</sup>Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. <sup>6</sup>Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. <sup>7</sup>Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA. <sup>8</sup>Department of Health Sciences Research, Mayo Clinic Cancer Center, Jacksonville, Florida 32224, USA. <sup>9</sup>Department of Radiation Oncology, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. <sup>10</sup>Department of Biostatistics & Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA. <sup>11</sup>Department of Cancer Biology, Mayo Clinic Cancer Center, Jacksonville, Florida. <sup>†</sup>Deceased. Correspondence and requests for materials should be addressed to F.H. (email: frederik.holst@uib.no) or A.D.C. (email: achernia@broadinstitute.org)



**Figure 1. Truncated *ESR1* amplifications in two metastatic endometrial carcinomas.** Dot plots of *ESR1* copy-numbers (y-axis) determined by GeneChip measurements (grey dots) of two metastatic endometrial carcinomas (above: #4, below #2) are shown on the left. Horizontal red lines indicate the segmented copy-number level of chromosomal positions (mega base pairs) on chromosome 6 (x-axis). Position of full length *ESR1* (vertical green lines) as well as *ESR1* exons 1-4 and 5-8 are indicated as green rectangles (see also Figure 2). Regarding FISH signals of *ESR1* (green) and centromere 6 (orange) within a tumor nucleus (blue) are shown on the right. FISH and regarding GeneChip copy-number data of 28 metastatic endometrial carcinomas are summarized in Appendix A. FISH analyses of these tumors are documented in Supplementary Optical Dataset S1.

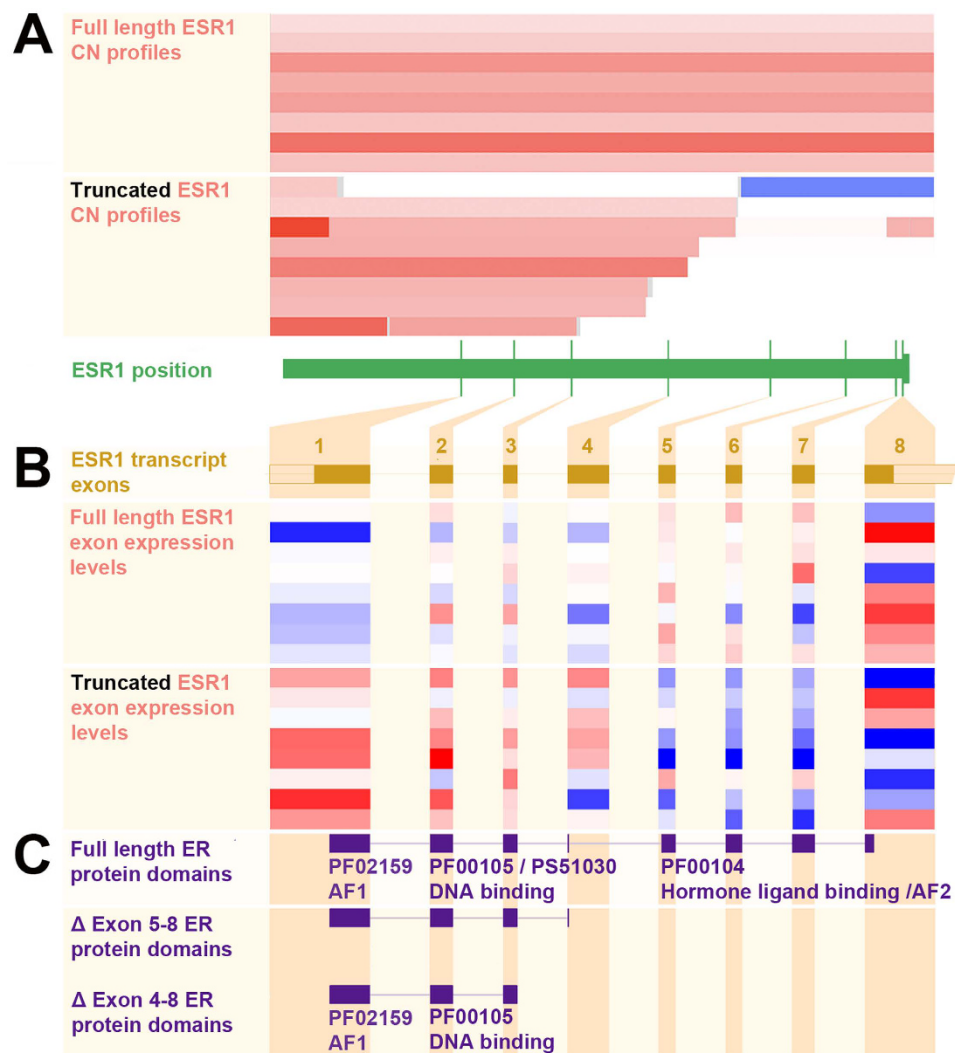
that alter the hormone-binding domain have been shown to generate hormone independence or resistance to anti-estrogen therapy in breast and endometrial cancers<sup>22–31</sup>, related genetic alterations could play a role for therapy outcome in primary endometrial carcinoma.

Recent studies identified mutations of *ESR1* in breast cancer that alter their hormone binding domain coding sequence, to be linked to endocrine therapy resistance in a metastatic setting<sup>26–28</sup>. One study by Li *et al.* even demonstrates an *ESR1* fusion in endocrine treatment resistant breast cancer, truncating the hormone-binding domain coding exons<sup>28</sup>, while a later study by Veeraraghavan *et al.* identified evidence for another type of recurrent ER $\alpha$ -altering gene fusions in this tumor type<sup>32</sup>. However, structural genetic alterations of *ESR1* have not been suggested to play a role in endometrial cancer carcinogenesis. Due to the potential importance of such *ESR1* alterations in endometrial cancer, we analyzed an tumor test subset of 29 primary endometrial cancers for somatic gene copy-number alterations (SCNA) and explored The Cancer Genome Atlas (TCGA)<sup>33</sup> for concerning SCNA and mRNA expression data of endometrial carcinoma.

## Results

Across a cancer study subset of 29 primary endometrial carcinomas that had gone on to metastasize, we characterized the copy-number changes by GeneChips and validated amplifications of *ESR1* in these cancers by fluorescence *in-situ* hybridization (FISH). The Pearson correlation of *ESR1* GeneChip copy numbers with FISH determined absolute average *ESR1* copy numbers per nucleus and average *ESR1* to centromere 6 (CEN6) ratios were  $r = 0.743$  ( $p < 0.001$ ) and  $r = 0.774$  ( $p < 0.001$ ) respectively (Appendix A, Fig. 1, Supplementary Figures S1 and S2, Supplementary Optical Dataset S1).

Four of these tumors exhibited focal *ESR1* amplification determined by GeneChips, of which two amplifications showed 3' truncations of *ESR1* ( $\Delta$  exon 6–8 or 7–8) that would remove the hormone-binding domain



**Figure 2. Truncated *ESR1* amplifications in TCGA endometrial carcinomas.** Log<sub>2</sub> *ESR1* copy number ratios of eight uterine corpus endometrial carcinomas with *ESR1* full-length amplification and eight carcinomas with *ESR1* truncating copy-number alterations are shown in horizontal bars (increased: red, normal/neutral: white, decreased: blue) (A). The corresponding heatmap of exon expression is estimated from RNA-Seq data (normalized relative higher: red, neutral: white, lower: blue) (B). Corresponding ER protein domains according to PROSITE (PS) and Pfam (PF) databases (<http://www.ebi.ac.uk/interpro/>) are shown in panel C (see <http://www.ensembl.org> for *ESR1* transcript variants).

(Appendix A, Fig. 1). We therefore explored the prevalence of *ESR1*-truncating amplifications across uterine corpus endometrial carcinoma within The Cancer Genome Atlas (TCGA)<sup>33</sup>.

**Hormone-binding domain truncated *ESR1* amplifications in primary endometrial cancers.** In the TCGA data subset of 539 endometrial carcinomas analyzed, we identified 88 (16.3%) cases with amplifications encompassing or overlapping *ESR1*. 46.6% of these were histologically defined serous and 75.0% of the tumors with *ESR1* amplification were clustered within the serous like copy-number high molecular weight subtype according to TCGA<sup>34</sup>. The *ESR1* amplifications were focal (less than half a chromosome arm in length) in 36 cases (6.7%) of tumors, and had a significantly higher rate of amplification than the genome-wide average ( $q = 5.75 \times 10^{-4}$ ). Mapping of the overlap between amplifications across tumors identified *ESR1* only as the most likely gene target (see methods).

These amplifications appeared to truncate the hormone-binding domain encoding region in seven cases (1.3% of the entire dataset; and 19.4% of cases with focal *ESR1* amplification) and to retain exons 1–4 or 1–3, encoding the n-terminal *ESR1* transactivation domain (AF1) and DNA-binding domains. Another case without *ESR1* amplification exhibited a heterozygous deletion of exons encoding the hormone-binding domain (Fig. 2), for a total apparent *ESR1* truncation rate of 1.5% over all tumors. In one additional TCGA case, we detected a hormone-binding domain (exons 4–8) truncating *ESR1*-*SYNE1* mRNA fusion (Appendix B). Eight of these

nine tumors were molecularly classified as being in the serous like copy-number high subgroup (4.3% of this subgroup)<sup>35</sup>.

**Association of *ESR1* exon copy numbers with mRNA expression.** The *ESR1* truncation events are associated with decreased mRNA expression of the truncated exons encoding the hormone-binding domain (exons 5–8) compared to the transactivation and DNA-binding domains (exons 1–4) ( $p < 0.001$ ) (Fig. 2 and Appendix C). We compared the normalized *ESR1* expression values estimated from RNA-Seq data for the eight tumors exhibiting amplified, truncated *ESR1* to those from eight tumors selected on the basis of exhibiting similarly focal *ESR1* amplifications that lack intragenic breakpoints. The average ratio between expression levels of exons 1–4 and 5–8 is 2.1-fold higher among truncated tumors relative to these controls ( $p = 0.003$ ). We also confirmed this relation after replacing the eight *ESR1*-amplified controls with all 545 tumors profiled by TCGA. In this comparison, the ratio of expression levels between exons 1–4 and 5–8 is 2.2-fold higher in *ESR1*-truncated tumors ( $p < 0.001$ ).

In contrast, TCGA breast cancers exhibit *ESR1* truncations on DNA-level less than half as often (7 of 1080; 0.65%) as observed in endometrial cancer and had increased expression of exons 1–2, but not of the full DNA-binding domain (Appendix D). These data suggest that the amplified truncations and associated mRNA profiles we describe in endometrial cancer are not frequent in breast cancer.

## Discussion

The gene truncations we report in endometrial carcinoma disrupt the hormone-binding domain encoding sequence of *ESR1*. Similarly, mRNA splice variants lacking one or more of exons 5–8, encoding the hormone-binding domain, have been described in normal<sup>35–38</sup> and malignant<sup>22,23,35–37,39</sup> breast as well as in normal<sup>22,40–45</sup> and malignant<sup>42–46</sup> endometrial tissue. Point mutations of the ligand binding domain encoding sequence of *ESR1* have also been described to occur in both breast and endometrial cancers<sup>25–27,30,47,48</sup>.

Both splice variants and point mutations involving the *ESR1* hormone-binding domain have been associated with hormone-independent ER $\alpha$  activity. The point mutations found in both breast and endometrial cancers have been shown to enable ligand-binding independent transcriptional activity<sup>26,30,48–50</sup> and have been related to acquired resistance to anti-estrogen therapy in breast cancer<sup>26–28</sup>. Excisions of exons 5 and 7 by alternative splicing have also been shown to constitutively activate ER $\alpha$ <sup>22,23,30</sup> and have been associated with hormone independent growth in both breast and endometrial cancer<sup>22–24,31</sup>. These findings raise the hypothesis that the *ESR1* truncations we report may also generate hormone-independent ER $\alpha$  activity.

In breast cancer, point mutations in the ligand-binding domain occur in 20–50% of tumors that have acquired resistance to anti-estrogen therapy<sup>26,27</sup> but only in 0.2% of primary cancers<sup>51</sup>. In endometrial cancer, however, point mutations and in-frame deletions altering the ligand binding domain occur in 2.8% of primary endometrial cancers<sup>26,51</sup>. Similarly the recurrent *ESR1* truncations we report appear to be much more frequent in primary endometrial carcinoma than in primary breast cancers.

Anti-estrogen therapy with estrogen antagonists or aromatase inhibitors is standard first-line treatment for ER $\alpha$ -positive breast cancers, but has been associated with only a low rate (~10%) of overall response among endometrial cancers<sup>13,16–18</sup> and is not a standard treatment for endometrial cancer<sup>3,4</sup>. In some cases, anti-estrogens such as Tamoxifen can even induce proliferation effect on endometrial cancer cells<sup>52,53</sup> and normal endometrial tissue<sup>54</sup> and increase the risk of endometrial carcinogenesis<sup>19–21</sup>. Splice variants of *ESR1* that alter the hormone-binding domain have been associated with ER $\alpha$  activation by Tamoxifen in endometrial cancer cells<sup>24</sup>. The effect of estrogen antagonists on ER $\alpha$  encoded by the truncated forms of *ESR1* that we have detected should also be tested, and all alterations of the *ESR1* ligand-binding domain should be evaluated as potential biomarkers of anti-estrogen therapy resistance. Conversely, the absence of such alterations should be evaluated as a biomarker of anti-estrogen sensitivity, potentially opening up a new therapeutic option for a subset of patients with endometrial cancer.

## Methods

**GeneChip analysis.** For our study subset of 29 primary endometrial tumors, gene copy-number data were determined by Affymetrix SNP 6.0 microarray analysis as described earlier<sup>55</sup>. GeneChip probe intensities are normalized across samples and circular binary segmentation is performed. Areas harboring germline CNVs are removed from the final segmented copy-number output. The range of birdseed call rates in this cohort was 92.6–99.3% with an average call rate of 97.1%. For TCGA copy-number data, level 3 segmented log<sub>2</sub> copy-number data were used in analysis. For both datasets, log<sub>2</sub> copy-number values are calculated as ratios relative to the genome wide average according to standard procedures<sup>56–59</sup>. These gene copy-number data were visualized using the IGV viewer software<sup>60</sup>. Linear gene level copy-number data were derived by GISTIC<sup>55,59</sup>. All TCGA DNA copy-number data (2015-06-01 stddata 2015-04-02 regular peel-off) can be accessed through the TCGA Copy Number Portal<sup>57</sup>.

**RNA-Seq analysis.** Reads per kilobase per million (RPKM)<sup>61</sup> RNA exon expression quantification values were normalized and RPKM 0 was assigned 0.1 (Appendices C+D). Exons were compared using inverted log<sub>2</sub> of normalized values. A two tailed Mann-Whitney-U-Test was applied to test for statistical significance of differences. P-values  $< 0.05$  were considered statistically significant. Paired-end RNA-seq fusion transcript analysis of TCGA RNA-sequencing data from 295 tumors to detect mRNA fusions was performed using SnowShoes-FTD as described earlier<sup>62–64</sup>. Parameters used to define a fusion transcript of high confidence were at least two unique fusion junction spanning split reads within the dataset and at least five encompassing reads<sup>65</sup>. RNA-Seq data were taken from the TCGA database <http://cancergenome.nih.gov>

**FISH analysis.** FISH was performed without RNase treatment as described earlier<sup>66</sup>. Pearson correlation coefficients and regarding p-values (two sided t-test) were generated using SPSS (Statistical Package of Social Science) version 20.0.0 applying standard bootstrapping. P-values < 0.05 were considered statistically significant.

**Tumor samples and DNA extraction.** This study has been approved by the Norwegian Data Inspectorate (961478-2), the Norwegian Social Science Data Services (15501) and the local Institutional Review Board (REKIII nr. 052.01) and the BROAD institute, MA, USA and methods were carried out in accordance with these approved guidelines. The 29 metastatic high grade primary tumor samples were obtained with documented informed consent in a patient based setting (Sept 2002–Sept 2012) from the Department of Obstetrics and Gynaecology, Section of Gynaecological Cancer, Haukeland University Hospital, Bergen, Norway. Biopsies were snap frozen in nitrogen and stored at minus 80 °C until DNA extraction. Tumor purity was assessed based on histology sections obtained by microtome prior to DNA extraction. DNA extraction was performed using samples with estimated tumor purity  $\geq 50\%$  as previously described<sup>7</sup>.

## References

- Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. Global cancer statistics, 2002. *CA Cancer J Clin* **55**, 74–108 (2005).
- Jemal, A. *et al.* Global cancer statistics. *CA Cancer J Clin* **61**, 69–90 (2011).
- Salvesen, H. B., Haldorsen, I. S. & Trovik, J. Markers for individualised therapy in endometrial carcinoma. *Lancet Oncol* **13**, e353–e361 (2012).
- Morice, P., Leary, A., Creutzberg, C., Abu-Rustum, N. & Darai, E. Endometrial cancer. *Lancet* **387**, 1094–1108 (2016).
- Zabransky, D. J. & Park, B. H. Estrogen receptor and receptor tyrosine kinase signaling: use of combinatorial hormone and epidermal growth factor receptor/human epidermal growth factor receptor 2-targeted therapies for breast cancer. *J Clin Oncol* **32**, 1084–1086 (2014).
- Yue, W., Yager, J. D., Wang, J. P., Jupe, E. R. & Santen, R. J. Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. *Steroids* **78**, 161–170 (2013).
- Li, Q. *et al.* Integrative eQTL-based analyses reveal the biology of breast cancer risk loci. *Cell* **152**, 633–641 (2013).
- Jongen, V. *et al.* Expression of estrogen receptor- $\alpha$  and - $\beta$  and progesterone receptor-A and -B in a large cohort of patients with endometrioid endometrial cancer. *Gynecol Oncol* **112**, 537–542 (2009).
- Burstein, H. J. *et al.* Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. *J Clin Oncol* **32**, 2255–2269 (2014).
- Wik, E. *et al.* Lack of estrogen receptor- $\alpha$  is associated with epithelial-mesenchymal transition and PI3K alterations in endometrial carcinoma. *Clin Cancer Res* **19**, 1094–1105 (2013).
- Silverberg, S. G. *et al.* Tumours of the uterine corpus: Epithelial tumors and related lesions. In *Pathology and Genetics of Tumours of the Breast and Female Genital Organs* (eds. Tavassoli, F. A. & Devilee, P.) 221–249 (IARC Press, Lyon, 2003).
- Rendina, G. M., Donadio, C., Fabri, M., Mazzoni, P. & Nazzicone, P. Tamoxifen and medroxyprogesterone therapy for advanced endometrial carcinoma. *Eur J Obstet Gynecol Reprod Biol* **17**, 285–291 (1984).
- Thigpen, T., Brady, M. F., Homesley, H. D., Soper, J. T. & Bell, J. Tamoxifen in the treatment of advanced or recurrent endometrial carcinoma: a Gynecologic Oncology Group study. *J Clin Oncol* **19**, 364–367 (2001).
- Pandya, K. J. *et al.* Megestrol and tamoxifen in patients with advanced endometrial cancer: an Eastern Cooperative Oncology Group Study (E4882). *Am J Clin Oncol* **24**, 43–46 (2001).
- Kokka, F., Brockbank, E., Oram, D., Gallagher, C. & Bryant, A. Hormonal therapy in advanced or recurrent endometrial cancer. *Cochrane Database Syst Rev* CD007926 (2010).
- Ma, B. B. *et al.* The activity of letrozole in patients with advanced or recurrent endometrial cancer and correlation with biological markers—a study of the National Cancer Institute of Canada Clinical Trials Group. *Int J Gynecol Cancer* **14**, 650–658 (2004).
- Rose, P. G. *et al.* A phase II trial of anastrozole in advanced recurrent or persistent endometrial carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol* **78**, 212–216 (2000).
- Lindemann, K. *et al.* Examestane in advanced or recurrent endometrial carcinoma: a prospective phase II study by the Nordic Society of Gynecologic Oncology (NSGO). *BMC Cancer* **14**, 68 (2014).
- American College of O. & Gynecologists Committee on Gynecologic, P. ACOG committee opinion. No. 336: Tamoxifen and uterine cancer. *Obstet Gynecol* **107**, 1475–8 (2006).
- Fisher, B. *et al.* Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J Natl Cancer Inst* **86**, 527–537 (1994).
- Bernstein, L. *et al.* Tamoxifen therapy for breast cancer and endometrial cancer risk. *J Natl Cancer Inst* **91**, 1654–1662 (1999).
- Fuqua, S. A. *et al.* Variant human breast tumor estrogen receptor with constitutive transcriptional activity. *Cancer Res* **51**, 105–109 (1991).
- Castles, C. G., Fuqua, S. A., Klotz, D. M. & Hill, S. M. Expression of a constitutively active estrogen receptor variant in the estrogen receptor-negative BT-20 human breast cancer cell line. *Cancer Res* **53**, 5934–5939 (1993).
- Horvath, G., Leser, G., Helou, K. & Henriksson, M. Function of the exon 7 deletion variant estrogen receptor  $\alpha$  protein in an estradiol-resistant, tamoxifen-sensitive human endometrial adenocarcinoma grown in nude mice. *Gynecol Oncol* **84**, 271–279 (2002).
- Herynk, M. H. & Fuqua, S. A. Estrogen receptor mutations in human disease. *Endocr Rev* **25**, 869–898 (2004).
- Robinson, D. R. *et al.* Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet* **45**, 1446–1451 (2013).
- Toy, W. *et al.* ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet* **45**, 1439–1445 (2013).
- Li, S. *et al.* Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep* **4**, 1116–1130 (2013).
- Fuqua, S. A., Gu, G. & Rechoum, Y. Estrogen receptor (ER)  $\alpha$  mutations in breast cancer: hidden in plain sight. *Breast Cancer Res Treat* **144**, 11–19 (2014).
- Weis, K. E., Ekena, K., Thomas, J. A., Lazennec, G. & Katzenellenbogen, B. S. Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Mol Endocrinol* **10**, 1388–1398 (1996).
- Lemieux, P. & Fuqua, S. The role of the estrogen receptor in tumor progression. *J Steroid Biochem Mol Biol* **56**, 87–91 (1996).
- Veeraraghavan, J. *et al.* Recurrent ESR1-CCDC170 rearrangements in an aggressive subset of oestrogen receptor-positive breast cancers. *Nat Commun* **5**, 4577 (2014).
- TCGA Research Network: The Cancer Genome Atlas (2010). <http://cancergenome.nih.gov/> Date of access: 21/07/2015.
- Cancer Genome Atlas Research, N. *et al.* Integrated genomic characterization of endometrial carcinoma. *Nature* **497**, 67–73 (2013).
- Poola, I. & Speirs, V. Expression of alternatively spliced estrogen receptor  $\alpha$  mRNAs is increased in breast cancer tissues. *J Steroid Biochem Mol Biol* **78**, 459–469 (2001).
- Pfeffer, U., Fecarotta, E. & Vidali, G. Coexpression of multiple estrogen receptor variant messenger RNAs in normal and neoplastic breast tissues and in MCF-7 cells. *Cancer Res* **55**, 2158–2165 (1995).

37. Leygue, E. R., Watson, P. H. & Murphy, L. C. Estrogen receptor variants in normal human mammary tissue. *J Natl Cancer Inst* **88**, 284–290 (1996).
38. Thomas, C. & Gustafsson, J. A. The different roles of ER subtypes in cancer biology and therapy. *Nat Rev Cancer* **11**, 597–608 (2011).
39. Daffada, A. A. *et al.* Exon 5 deletion variant estrogen receptor messenger RNA expression in relation to tamoxifen resistance and progesterone receptor/pS2 status in human breast cancer. *Cancer Res* **55**, 288–293 (1995).
40. Daffada, A. A. & Dowsett, M. Tissue-dependent expression of a novel splice variant of the human oestrogen receptor. *J Steroid Biochem Mol Biol* **55**, 413–421 (1995).
41. Springwald, A. *et al.* Identification of novel transcript variants of estrogen receptor alpha, beta and progesterone receptor gene in human endometrium. *Endocrine* **37**, 415–424 (2010).
42. Skrzypczak, M. *et al.* Molecular profiling of estrogen receptor alpha and progesterone receptor transcript variants in endometrial cancer. *Steroids* **104**, 122–128 (2015).
43. Rice, L. W., Jazaeri, A. A. & Shupnik, M. A. Estrogen receptor mRNA splice variants in pre- and postmenopausal human endometrium and endometrial carcinoma. *Gynecol Oncol* **65**, 149–157 (1997).
44. Hu, C., Hyder, S. M., Needleman, D. S. & Baker, V. V. Expression of estrogen receptor variants in normal and neoplastic human uterus. *Mol Cell Endocrinol* **118**, 173–179 (1996).
45. Hirata, S. *et al.* Presence of alternatively spliced-estrogen receptor mRNA variants in normal human uterine endometrium and endometrial cancer. *Endocr J* **42**, 289–293 (1995).
46. Jazaeri, O., Shupnik, M. A., Jazaeri, A. A. & Rice, L. W. Expression of estrogen receptor alpha mRNA and protein variants in human endometrial carcinoma. *Gynecol Oncol* **74**, 38–47 (1999).
47. Assikis, V. J., Bilimoria, M. M., Muenzner, H. D., Lurain, J. R. & Jordan, V. C. Mutations of the estrogen receptor in endometrial carcinoma: evidence of an association with high tumor grade. *Gynecol Oncol* **63**, 192–199 (1996).
48. Zhang, Q. X., Borg, A., Wolf, D. M., Oesterreich, S. & Fuqua, S. A. An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer Res* **57**, 1244–1249 (1997).
49. Thomas, C. & Gustafsson, J. A. Estrogen receptor mutations and functional consequences for breast cancer. *Trends Endocrinol Metab* **26**, 467–476 (2015).
50. Skafar, D. F. Formation of a powerful capping motif corresponding to start of “helix 12” in agonist-bound estrogen receptor-alpha contributes to increased constitutive activity of the protein. *Cell Biochem Biophys* **33**, 53–62 (2000).
51. Gao, J. *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* **6**, pl1 (2013).
52. Satyaswaroop, P. G., Zaino, R. J. & Mortel, R. Estrogen-like effects of tamoxifen on human endometrial carcinoma transplanted into nude mice. *Cancer Res* **44**, 4006–4010 (1984).
53. Gottardis, M. M., Robinson, S. P., Satyaswaroop, P. G. & Jordan, V. C. Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. *Cancer Res* **48**, 812–815 (1988).
54. Decensi, A. *et al.* Effect of tamoxifen on endometrial proliferation. *J Clin Oncol* **14**, 434–440 (1996).
55. Mermel, C. H. *et al.* GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol* **12**, R41 (2011).
56. Zack, T. I. *et al.* Pan-cancer patterns of somatic copy number alteration. *Nat Genet* **45**, 1134–1140 (2013).
57. TCGA Research Network: TCGA Copy Number Portal (2010). <http://www.broadinstitute.org/tcga/home> Date of access: 21/07/2015.
58. TCGA. Integrated genomic characterization of endometrial carcinoma. *Nature* **497**, 67–73 (2013).
59. Beroukhi, R. *et al.* The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899–905 (2010).
60. Robinson, J. T. *et al.* Integrative genomics viewer. *Nat Biotechnol* **29**, 24–26 (2011).
61. Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L. & Wold, B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods* **5**, 621–628 (2008).
62. Asmann, Y. W. *et al.* A novel bioinformatics pipeline for identification and characterization of fusion transcripts in breast cancer and normal cell lines. *Nucleic Acids Res* **39**, e100 (2011).
63. Asmann, Y. W. *et al.* Detection of redundant fusion transcripts as biomarkers or disease-specific therapeutic targets in breast cancer. *Cancer Res* **72**, 1921–8 (2012).
64. Norton, N. *et al.* Gene expression, single nucleotide variant and fusion transcript discovery in archival material from breast tumors. *PLoS One* **8**, e81925 (2013).
65. Smallridge, R. C. *et al.* RNA sequencing identifies multiple fusion transcripts, differentially expressed genes, and reduced expression of immune function genes in BRAF (V600E) mutant vs BRAF wild-type papillary thyroid carcinoma. *J Clin Endocrinol Metab* **99**, E338–E347 (2014).
66. Moelans, C. B., Holst, F., Hellwinkel, O., Simon, R. & van Diest, P. J. ESR1 Amplification in Breast Cancer by Optimized RNase FISH: Frequent but Low-Level and Heterogeneous. *PLoS One* **8**, e84189 (2013).

## Author Contributions

F.H. and A.D.C. designed and conducted the analyses, and prepared the manuscript, together with support from E.A.H., W.J.G., A.T., S.E.S., Y.W.A., P.G., J.T., B.M.N., E.A.T., M.M., R.B. and H.B.S.

## Additional Information

**Supplementary information** accompanies this paper at <http://www.nature.com/srep>

**Competing financial interests:** Frederik Holst has royalty interest associated with intellectual property of ZytoVision GmbH concerning patent US8101352B2 “Detection of ESR1 Amplification in Breast Cancer” and according EU patent application. Mathew Meyerson and Andrew Cherniack receive research support from Bayer AG.

**How to cite this article:** Holst, F. *et al.* Recurrent hormone-binding domain truncated *ESR1* amplifications in primary endometrial cancers suggest their implication in hormone independent growth. *Sci. Rep.* **6**, 25521; doi: 10.1038/srep25521 (2016).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

# SCIENTIFIC REPORTS

**OPEN**

## **Corrigendum: Recurrent hormone-binding domain truncated *ESR1* amplifications in primary endometrial cancers suggest their implication in hormone independent growth**

Frederik Holst, Erling A. Hoivik, William J. Gibson, Amaro Taylor-Weiner, Steven E. Schumacher, Yan W. Asmann, Patrick Grossmann, Jone Trovik, Brian M. Necela, E. Aubrey Thompson, Matthew Meyerson, Rameen Beroukhim, Helga B. Salvesen & Andrew D. Cherniack

*Scientific Reports* 6:25521; doi: 10.1038/srep25521; published online 10 May 2016; updated on 24 June 2016

In the original version of this Article, there were errors in Affiliation 2 which was incorrectly given as ‘KG Jebsen Center for Precision Medicine in Gynecologic Cancer, Department of Gynecology and Obstetrics, Haukeland University Hospital Bergen, Norway’. The correct affiliation is listed below:


‘Department of Gynecology and Obstetrics, Haukeland University Hospital Bergen, Norway’.

This error has now been corrected in the PDF and HTML versions of the Article.



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

# SCIENTIFIC REPORTS



OPEN

## Corrigendum: Recurrent hormone-binding domain truncated *ESR1* amplifications in primary endometrial cancers suggest their implication in hormone independent growth

Frederik Holst, Erling A. Hoivik, William J. Gibson, Amaro Taylor-Weiner, Steven E. Schumacher, Yan W. Asmann, Patrick Grossmann, Jone Trovik, Brian M. Necela, E. Aubrey Thompson, Matthew Meyerson, Rameen Beroukhim, Helga B. Salvesen & Andrew D. Cherniack

*Scientific Reports* 6:25521; doi: 10.1038/srep25521; published online 10 May 2016; updated on 30 June 2017

This Article contains errors in the frequencies of *ESR1* amplifications concerning uterine corpus endometrioid carcinoma (UCEC) in TCGA, which were incorrectly given as 88 cases (16.3%) and 36 cases (6.7%) for overall and focal amplifications respectively. These numbers represent the regarding amplification frequencies of partial *ESR1* sequences that manifest the GISTIC peak.

The correct frequencies of *ESR1* amplification in UCEC of TCGA are 90 cases (16.7%) and 39 cases (7.2%) for overall and focal amplification respectively. Accordingly, the seven cases with *ESR1* amplifications that appear to truncate the hormone-binding domain encoding region manifest 17.9% instead of 19.4% of cases with focal amplification.

In addition, the GISTIC q-value for *ESR1* amplification is incorrectly given as “ $q = 5.75 \times 10^{-4}$ ”. The correct term and number for this value is “residual  $q = 2.29 \times 10^{-4}$  after ‘GISTIC peel-off’”.

As a result, in the Results section under subheading ‘Hormone-binding domain truncated *ESR1* amplifications in primary endometrial cancers.’

“In the TCGA data subset of 539 endometrial carcinomas analyzed, we identified 88 (16.3%) cases with amplifications encompassing or overlapping *ESR1*. 46.6% of these were histologically defined serous and 75.0% of the tumors with *ESR1* amplification were clustered within the serous like copy-number high molecular subtype according to TCGA. The *ESR1* amplifications were focal (less than half a chromosome arm in length) in 36 cases (6.7%) of tumors, and had a significantly higher rate of amplification than the genome-wide average ( $q = 5.75 \times 10^{-4}$ ). Mapping of the overlap between amplifications across tumors identified *ESR1* only as the most likely gene target (see methods).

These amplifications appeared to truncate the hormone-binding domain encoding region in seven cases (1.3% of the entire dataset; and 19.4% of cases with focal *ESR1* amplification) and to retain exons 1–4 or 1–3, encoding the n-terminal *ESR1* transactivation domain (AF1) and DNA-binding domains. Another case without *ESR1* amplification exhibited a heterozygous deletion of exons encoding the hormone-binding domain (Fig. 2), for a total apparent *ESR1* truncation rate of 1.5% over all tumors. In one additional TCGA case, we detected a hormone-binding domain (exons 4–8) truncating *ESR1-SYNE1* mRNA fusion (Appendix B). Eight of these



nine tumors were molecularly classified as being in the serous like copy-number high subgroup (4.3% of this subgroup)”.

should read:

“In the TCGA data subset of 539 endometrial carcinomas analyzed, we identified 90 (16.7%) cases with amplifications encompassing or overlapping *ESR1*. 45.6% of these were histologically defined serous and 75.0% of the tumors with *ESR1* amplification were clustered within the serous like copy-number high molecular subtype according to TCGA. The *ESR1* amplifications were focal (less than half a chromosome arm in length) in 39 cases (7.2%), and had a significantly higher rate of amplification than the genome-wide average (residual  $q = 2.29 \times 10^{-4}$  after “GISTIC peel-off”). Mapping of the overlap between amplifications across tumors identified *ESR1* only as the most likely gene target (see methods).

These amplifications appeared to truncate the hormone-binding domain encoding region in seven cases (1.3% of the entire dataset; and 17.9% of cases with focal *ESR1* amplification) and to retain exons 1–4 or 1–3, encoding the n-terminal *ESR1* transactivation domain (AF1) and DNA-binding domains. Another case without *ESR1* amplification exhibited a heterozygous deletion of exons encoding the hormone-binding domain (Fig. 2), for a total apparent *ESR1* truncation rate of 1.5% over all tumors. In one additional TCGA case, we detected a hormone-binding domain (exons 4–8) truncating *ESR1-SYNE1* mRNA fusion (Appendix B). Eight of these nine tumors were molecularly classified as being in the serous like copy-number high subgroup (4.3% of this subgroup)”.



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

© The Author(s) 2017