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ESR1 gene amplification: another mechanism regulating the cellular levels of ERα

Ke-Da Yu and Zhi-Ming Shao

We read with great interest the Review (The different roles of ER subtypes in cancer biology and therapy. *Nature Rev. Cancer* **11**, 597–608 (2011))[1] by Thomas and Gustafsson, in which the authors addressed the mechanisms of action and regulation of oestrogen receptors (ERs), the distinct biological effect of ER isoforms, and the roles of ERs in cancer prognosis and targeted therapies. Regarding the regulation of cellular levels of ERs, the authors systemically stated the potential mechanisms, including promoter methylation, post-transcriptional regulation by specific microRNAs and proteasome-mediated degradation of ER proteins. Although the aforementioned mechanisms are the main pathways of ER regulation, the complete mechanisms of ER upregulation are still not fully understood. One mechanism that may lead to the overexpression of a given gene in neoplastic cells is gene amplification, and *ESR1* gene amplification has been observed in some cancer cells[2]. We believe that this issue, which is not mentioned in the Review, deserves to be discussed when illustrating the mechanisms of upregulation of ERα.

It has recently been reported that expression of ERα, but not expression of ERβ, is driven in some cases by *ESR1* amplification[2]. Holst *et al.* used tissue microarray analysis of more than 2,000 samples to demonstrate that 20.6% of breast cancers contained *ESR1* amplification[2]; Burkhardt *et al.* showed that amplification rates of *ESR1* in ductal carcinoma *in situ* (DCIS), DCIS with invasive cancer and the invasive component did not differ significantly from one another, with rates of 19.0%, 24.1% and 21.3%, respectively[3]. More interestingly, almost all tumours with *ESR1* amplification showed ERα protein overexpression[2]. It is therefore not surprising that *ESR1* amplification in breast cancers was found to be associated with improved survival in women who had received adjuvant tamoxifen. In patients with ER-positive disease who received tamoxifen monotherapy, survival was indeed shown to be longer in patients who had *ESR1* amplification than in patients who did not have *ESR1* amplification[2]. Another Japanese report also showed that *ESR1* amplification, which was found in 22.6% of samples by three-dimensional fluorescence *in situ* hybridization (FISH) assay, strongly correlates with higher expression levels of ERα, and that patients with *ESR1* amplification in tumours apparently experience longer disease-free survival than those without *ESR1* amplification[4]. These observations consistently suggest that *ESR1* amplification is helpful in selecting patients who may potentially benefit from endocrine therapy. Besides breast cancer, *ESR1* amplification occurs in more than 20% of endometrial carcinomas (established using FISH[5]) but it rarely occurs (2%) in ovarian cancer[6].

However, the relatively high prevalence of *ESR1* amplification in breast cancer (about 20%) has been challenged by several groups, who found *ESR1* amplification in only approximately 1–5% of breast cancers[7–10] (TABLE 1). One potential explanation for this is that independent investigators used a variety of different techniques[11]. The high prevalence of *ESR1* amplification is mainly observed by FISH rather than by comparative genomic hybridization (CGH) or by quantitative-PCR. It is likely that contamination of tumour DNA with normal DNA (from the stroma, for example) is not only a challenge for detecting low-level amplicons in array CGH study, but is also a major drawback in quantitative-PCR. It is also difficult to accurately distinguish multiple small signals from a large confluent signal, given the small size of the *ESR1* amplicon in breast cancer. The distance between the signals is often smaller than the diameter of one FISH signal. Such clusters are difficult to count, although the tumour appears to be amplified at first sight during a visual inspection. As a result, most *ESR1*-amplified tumours are considered as unamplified if ERBB2 criteria are applied. New criteria for estimating the *ESR1* gene copy number need to enable a more reliable identification of amplified cancers than identification by classical counting.

Because alteration of ER expression is an important step in the development and progression of hormone-related cancers, and because it influences cancer response to

Table 1 | **Available data for *ESR1* (at locus 6q25.1) amplification in cancer**

Cancer type	Method	Total number of cases	Number of cases of *ESR1* amplification	Number of cases of *ESR1* copy number gain	Refs
Breast cancer	Array CGH	148	1 (1%)	2 (1%)	12
Breast cancer	Array CGH	31	3 (10%)	1 (3%)	13
Breast cancer	Array CGH	391	4 (1%)	18 (5%)	7
Breast cancer	Array CGH	68	0 (0%)	5 (7%)	8
Breast cancer	Array CGH	341	3 (1%)	ND	10
Breast cancer	Array CGH	70	3 (4.3%)	ND	9
Breast cancer	CISH	148	2 (1.35%)	ND	9
Breast cancer	Quantitative PCR	35	2 (5.7%)	2 (5.7%)	9
Breast cancer	Array CGH	22	1 (4.5%)	ND	11
Breast cancer	FISH	1,739	358 (20.6%)	266 (15.3%)	2
Breast cancer	Array CGH	274	0 (0%)	5 (2%)	14
Breast cancer*	FISH	108	23 (21.3%)	ND	3
Breast cancer	FISH	133	30 (22.6%)	15 (11.3%)	4
Breast cancer	MLPA	104	2 (2%)	15 (14%)	15
Endometrial cancer	FISH	176	40 (22.7%)	10 (5.7%)	5
Ovarian cancer	FISH	243	5 (2.1%)	ND	6

CGH, comparative genomic hybridization; CISH, chromogenic *in situ* hybridization; FISH, fluorescence *in situ* hybridization; MLPA multiplex ligation-dependent probe amplification; ND, not determined.
*Invasive component of breast ductal carcinoma *in situ*.

endocrine therapy[1], *ESR1* amplification leading to upregulation of ERα expression might have clinical importance, and further well-designed investigations are needed to resolve this issue.

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Competing interests statement
The authors declare no competing financial interests.