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The relevance of phosphorylated forms of estrogen receptor in human breast cancer *in vivo*☆

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ABSTRACT

Estrogen receptor (ER)α activity is regulated by phosphorylation at several sites. Recently several antibodies specific for individual phosphorylated sites within ERα have became available. Validation and use of these antibodies suggests that several forms of phosphorylated ERα can be detected in multiple ER+ human breast tumor samples, thus providing relevance for investigating the regulation and function of phosphorylated ERα in human breast cancer. Generally, the phosphorylated ERα isoforms are associated with parameters that suggest that they are markers of an intact estrogen dependent signaling pathway and better clinical outcome with respect to tamoxifen therapy. Profiling of phosphorylated ERα may provide better biomarkers of endocrine therapy response over and above those currently available.

1. Introduction

The estrogen status of human breast tumors is an important biomarker with respect to both prognosis and prediction of treatment response [1]. The latter is probably of more practical importance and ER+ status predicts for responsiveness to endocrine therapies such as tamoxifen, other selective ER modulators (SERMs) and aromatase inhibitors. This is because of the central role of ER in estrogen signaling [2]. However, only about 50% of patients whose tumors are ER+ will respond to tamoxifen treatment and therefore display *de novo* resistance to tamoxifen and of those that originally respond, many will eventually progress on tamoxifen treatment having acquired tamoxifen resistance [3]. The need for more precise markers of treatment response and other targets for endocrine therapies has prompted us to investigate phosphorylated forms of ER in this regard.

ERs are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors that regulate transcription by recruiting co-activator proteins via estrogen independent and estrogen dependent activation function regions (AF-1 and AF-2 respectively) [4]. Phosphorylation, a well investigated post-translational modification event, that regulates subcellular localization, dimerization, DNA binding and transcriptional activity of nuclear receptors is an important mechanism for regulating ER activity [5,6]. Currently ER status in breast tumors only reflects an assessment of ERα expression [7], so it is the phosphorylation of ERα that will be the focus of this discussion.

2. Studies of P-ERα in human breast cancer biopsy samples

Multiple phosphorylation sites on ERα have been identified [5,8–11] and potential functional roles have been studied using, most often, mutated receptors expressed in cell culture models [5,12]. Little, if any, *in vivo* studies have been published. Since ERα can be a substrate of several kinases activated by growth factor/growth factor receptor driven pathways in breast cancer, and growth factor receptor pathways are often upregulated and/or abnormally regulated during breast cancer progression, the ligand independent activation of ERα has been hypothesized to be a mechanism by which resistance to endocrine therapies in breast cancer may occur [13]. One way to gain insights as to potential roles *in vivo* has been to measure the expression of the phospho-ERα (P-ERα) and determine if there are any significant associations with known parameters of prognosis and treatment outcome [14,15].

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Antibodies to phospho-specific sites on ERα i.e. P-serine^{118}-ERα (P-S118) and P-serine^{167}-ERα (P-S167) have recently become commercially available. As a result there are now a few studies in which the expression of one or both of these two P-ERα sites has been determined in cohorts of breast tumor biopsy samples and the results published [10,14–20]. As well some studies using custom antibodies to other sites of P-ERα have been published recently [9,10]. A list of the published studies is shown in Table 1. In some of these studies associations with other histopathological markers, clinical outcome and specific kinases have been found. Differences in association of these phospho-epitopes with established parameters in breast cancer and clinical outcome due to endocrine therapies have been found and will be discussed below.

3. Validation of antibodies

3.1. Immunohistochemistry (IHC) and Western blotting

In most cases the specificity of the phospho-specific antibodies has been done by Western blot of extracts from cell lines transiently transfected with wild-type or serine to alanine mutant expression plasmids. However, this is really not rigorous enough when it comes to either Western blotting of extracts from human breast cancer biopsies or for IHC. And the lack of rigorous validation of antibody specificity for the application to be used, may be a significant factor in the contrasting conclusions that have been made.

Validation for IHC should include positive predominantly nuclear staining in ER positive and not ER negative tumors, together with loss of signal by pre-absorption of the antibody with excess specific phospho-peptide used to generate the antibody, no loss of signal with excess non-phospho-peptide nor excess irrelevant phospho-peptide in known positive tumors, taking into account the known tumor cell heterogeneity found in breast biopsy samples. Some of the studies but not all of the publications have reported these types of validation [10,17,19,20]. Those that have performed extensive validation are marked with an asterisk in Table 1.

Some studies have also provided data in which Western blots of extracts from fresh frozen human breast cancer biopsy samples using P-ERα specific antibodies have detected appropriately sized bands [20,21]. In our hands, we find that multiple bands can often be found using Western blots of breast biopsy extracts and therefore we have also used immunoneutralization studies to confirm the specificity of the bands. Examples of the data are shown in Fig. 1. Using tumors that were IHC positive for P-S167 and for which frozen material was available, extracts were made and subjected to Western blotting. In the positive tumors an appropriately sized band disappeared after immuno-neutralization with the phospho-specific peptide (Fig. 1A, right top panels) but not with the non-phosphorylated peptide (Fig. 1A, right bottom panels). Similarly, we identified a specifically immuno-neutralizable protein for P-S118 in breast tumors that was positive by IHC but not in a tumor that was negative for staining (Fig. 1B, left panels, non-absorbed antibodies; right panels pre-absorbed antibodies). It should be noted that the upper band of the doublet that is seen with the P-S118 antibody is immuno-neutralized and specific, while the lower band of the doublet is not neutralized and therefore is considered non-specific. This lower non-specific band has been previously observed in extracts from MCF-7 cells using the same antibody [12,22].

Table 1
List of published studies of the determination of P-ERα expression in human breast cancer biopsy samples.

P-ERα	Number of cases	Reference
P-S118	45	Murphy et al. (2004)
P-S118	113	Murphy et al. (2004)
P-S118	Not known	Gee et al. (2005)
P-S118	75	Yamashita et al. (2005)
P-S167	75	Yamashita et al. (2005)
P-S118	301	Sarwar et al. (2006)*
P-S118	40	Weitsman et al. (2006)
P-S118	279	Bergqvist et al. (2006)
P-S118	290	Jiang et al. (2007)*
P-S167	290	Jiang et al. (2007)
P-S305	377	Holm et al. (2008)*
P-S118	278	Yamashita et al. (2008)
P-S167	278	Yamashita et al. (2008)
P-S118	370	Skliris et al. (in press)*
P-S104/106	301	Skliris et al. (in press)*
P-S167	400	Skliris et al. (in press)*
P-S282	374	Skliris et al. (in press)*
P-S294	410	Skliris et al. (in press)*
P-T311	406	Skliris et al. (in press)*
P-S559	380	Skliris et al. (in press)*

* Values that have performed extensive validation.

3.2. Epitope stability

Stability of the phospho-epitopes under the collection and storage conditions of the tumor tissue may also be a problem and contribute to differences in study conclusions. We have been some of the first to try and determine the effect of time to collection on expression of RNA, proteins and phospho-epitopes within the ER in human breast tumor biopsy samples [10,23]. Our data suggest that longer times of tissue collection to processing and storage result in decreased ER and PR levels as determined by ligand binding assays (LBA) [10,23] but have little effect on total ER protein measured by IHC [10]. We also used a subset of these timed collection samples to do preliminary studies to investigate effects of time to collection on P-ERα stability [10]. We examined P-S118 and P-S167, and while there was a trend to decreased expression as determined by H-score in the ≥30 min compared to the <30 min collection groups, the differences were not statistically significant. However, this may be due to the small sample size used. Furthermore, it is possible that different phosphorylation sites may be more or less stable than the ones we have specifically measured in our study. However, it is still unclear how the extent of the variability of epitope expression due to time of tissue collection, might be significant in comparison to the scale of the biological differences amongst biospecimens [23], and several studies using stored human breast biopsy samples have found biologically plausible correlations of P-ERα with known important clinical and pathological parameters in human breast tumors as reviewed in this current manuscript (see Tables 1 and 2).

4. Expression of P-ERα in human breast cancer biospy samples and relationship to clinical parameters and outcome

Insights into the putative role of gene expression in human breast cancer *in vivo* is often obtained by investigating the relationship of expression in retrospectively collected cohorts of samples, to other biomarkers of prognosis and treatment response as well as to clinical outcomes such as relapse free survival (RFS) and overall survival (OS). A summary of such data currently published with regard to phosphorylated forms of ERα is presented in Table 2. As is frequently the case with such studies, quite contradictory results are often found, variably due to the relatively small numbers in the cohorts studied, differences in scoring and quantifying expression, differences in definitions of positivity and negativity, differences in the characteristics of the patient populations studied, technical issues etc. However, some common themes are emerging especially with regard to P-S118 and P-S167 which have been the major focus to date. In particular the detection of P-ERα is generally associated

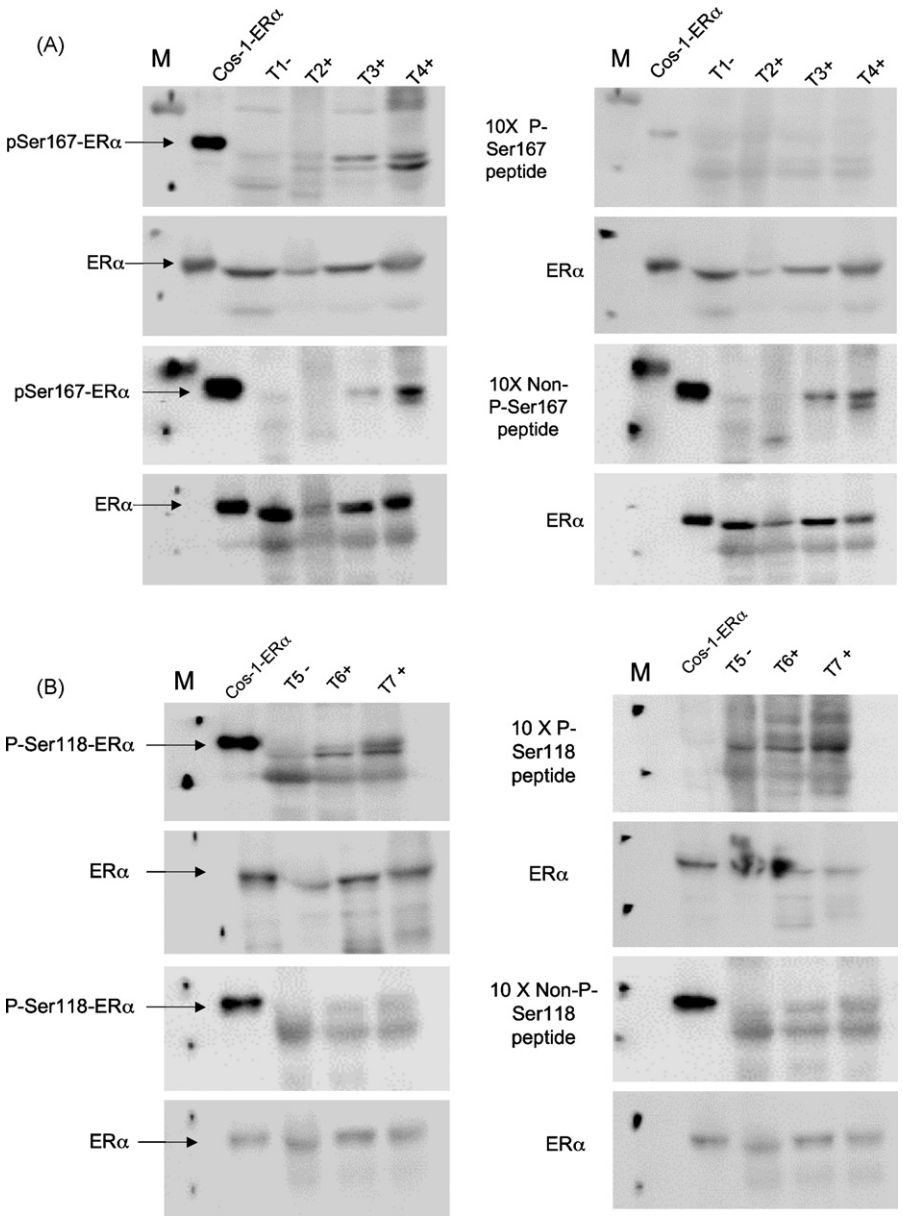

Fig. 1. Expression of ERα phosphorylated at P-S167 and P-S118 in human breast tumor extracts. (A) The left panels show extracts from breast tumors (T) that were positive (+) or negative (−) for P-S167-ERα IHC, analyzed by Western blotting and visualized with the anti-P-S167-ERα or total ERα antibodies. The right panel shows the same extracts analyzed with the P-specific antibody pre-absorbed with excess phosphorylated peptide (10× P-S167-ERα, right top panel) or the same extracts analyzed with the antibody pre-absorbed with excess non-phosphorylated peptide (10× non-P-S167-ERα) or the same blots incubated with antibody to total ERα. (B) Extracts from breast tumors that were positive or negative for P-S118-ERα IHC, analyzed by Western blotting and visualized with the anti-P-S118-ERα antibodies. The right panel shows the same extracts analyzed with the antibody pre-absorbed with excess phosphorylated peptide; the second from bottom panel shows the same extracts analyzed with the antibody pre-absorbed with excess non-phosphorylated peptide. 100 μg protein from breast tumor extracts were analysed by immunoblotting. Positive controls obtained from transiently transfected COS-1 cells were also included. Total ERα levels for each extract are shown on the bottom panel for both A and B using ERα monoclonal antibody (clone 6F11, Novocastra, Newcastle, UK). N.B. In tumor extracts both the phosphorylated ERα and the total ERα run slightly below the equivalent immunoreactive protein in cell line extracts.

with more differentiated, less aggressive tumor characteristics and features of an intact estrogen responsive signaling pathway. This is consistent with either P-S167 or P-S118 positivity being most often associated with a better clinical outcome to tamoxifen therapy in retrospectively collected cohorts [14,19,24,25]. We have confirmed our previously published results [14] in a larger cohort of patients representing over 300 cases, where expression of P-S118 was associated with a significantly better clinical outcome on tamoxifen therapy (Fig. 2A and B). As well, we observed that in this latter cohort detection of P-S167 was associated with a trend towards better clinical outcome on tamoxifen (Fig. 2C and D). This latter result supports the earlier results published by Jiang et al. [19] and Yamashita et al. [25]. In our cohort, the results also show that measurement of PR either by IHC or LBA (Fig. 2E–H) is still a stronger predictor of RFS and OS on tamoxifen therapy than the phosphorylated forms of ERα. Interestingly, however, our previous results showed that addition of P-ERα to PR may further improve the prediction of response to endocrine therapy [14].

The results also suggest that in primary breast tumors detection of P-ERα, in particular, P-S118 and P-S167 but perhaps not P-S305 [20], is unlikely to be the cause of *de novo* tamoxifen resistance due to over-expression of growth factors such as HER2 [13,26]. Some studies have found a weak positive association of HER2 expression and P-S118 [19,25], however the one study, in which the

Table 2
P-ERα expression and relationship to clinical outcome on tamoxifen therapy and other biomarkers in human breast cancer.

P-ERα	Clinical outcome to tamoxifen	Association with other biomarkers	Number of cases	Reference
P-S118		Negative with grade ($P = 0.023$)	45	Murphy et al. (2004)
P-S118	+P-ERα longer DFS ($P = 0.0018$)	Positive with PR ($P = 0.012$)	113	Murphy et al. (2004)
P-S118	+P-ERα longer TTP $P < 0.009$		Not known	Gee et al. (2005)
P-S118		Positive with p-S167 ($P < 0.0001$)	75	Yamashita et al. (2005)
P-S118		Positive PRa ($P < 0.0001$)	75	Yamashita et al. (2005)
P-S118		Higher in lymph node mets vs.1^0 ($P = 0.0098$)	75	Yamashita et al. (2005)
P-S118	+P-ERα longer DFS $P = 0.033$	Positive with p-S118 ($P < 0.0001$)	75	Yamashita et al. (2005)
P-S118		Positive with PRa ($P = 0.0007$)	75	Yamashita et al. (2005)
P-S118		Negative with grade ($P < 0.001$)	301	Sarwar et al. (2006)
P-S118	No association with outcome	Positive with PR ($P = 0.09$)	301	Sarwar et al. (2006)
P-S118		Positive with PR ($P < 0.01$)	279	Bergqvist et al. (2006)
P-S118		Negative with grade ($P = 0.009$)	290	Jiang et al. (2007)
P-S167	+P-ERα longer DFS ($P = 0.006$)	Positive with p-S118 ($P < 0.0005$)	290	Jiang et al. (2007)
P-S167	+P-ERα better OS ($P = 0.023$)	Negative with tumor size ($P = 0.002$)	290	Jiang et al. (2007)
P-S305	Negative for P-ER more Tam responsive ($P = 0.01$)	Positive P-ER with high grade (0.032)	377	Holm et al. (2008)
P-S305		Positive P-ER With high MI ($P = 0.009$)	377	Holm et al. (2008)
P-S305		Positive P-ER with small tumors ($P = 0.025$)	377	Holm et al. (2008)
P-S118	Low P-ER with better DFS ($P = 0.0003$), better OS ($P = 0.0007$)	Positive with p-S167 ($P < 0.0001$)	240	Yamashita et al. (2008)
P-S167	High P-ER with better DFS ($P = 0.0002$), better OS ($P = 0.0016$)	Positive with p-S118 ($P < 0.0001$)	210	Yamashita et al. (2008)

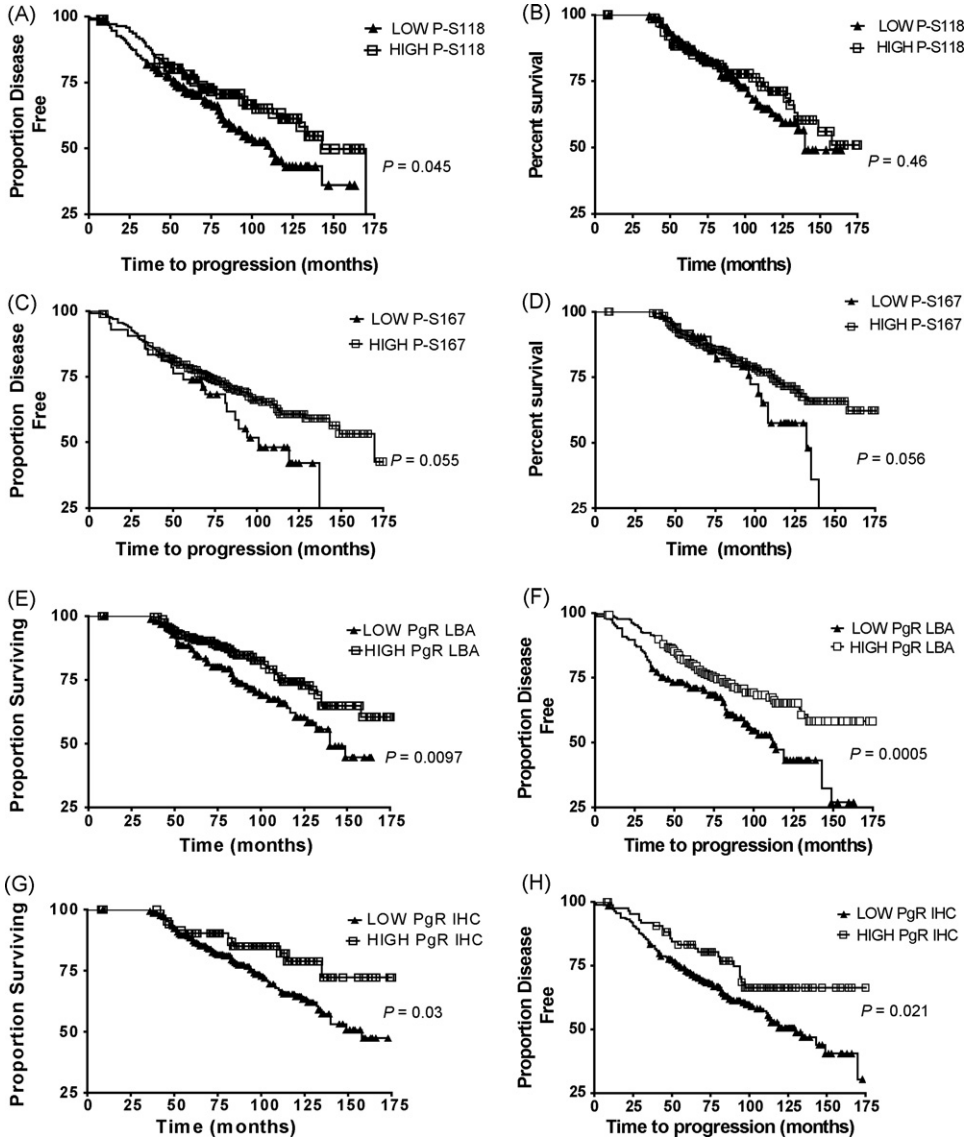

Fig. 2. Kaplan–Meier graphs for proportion disease free (RFS, relapse free survival-time to progression) and proportion/percent surviving (OS, overall survival) for patients treated with tamoxifen therapy. P-S118 protein expression in breast cancer study cohort (A and B); P-S167 protein expression in breast cancer study cohort (C and D); PR expression by LBA (E and F) and PR expression by IHC (G and H). Symbols on the graph lines represent censored data, *P* values are given for log-rank tests. P-Ser118-ERα positive/high defined by IHC-score >0 (48% positive), $n = 336$. P-Ser167-ERα positive/high defined by IHC score >0 (43% positive), $n = 381$. PR LBA positive/high defined as >20 fmol/mg protein, $n = 417$. PR IHC positive/high defined as IHC score >20, $n = 367$.

Table 3
Associations of P-S118-ERα and P-S167-ERα with active kinases in human breast tumor samples.

P-ERα	Active Kinase	Relationship	Reference
P-S118	P-MAPK/ERK1/2	Positive association $P < 0.0001$	Murphy et al. (2004) [1]
P-S118	P-MAPK/ERK1/2	Positive association $P < 0.001$	Sarwar et al. (2006) [2]
P-S118	P-MAPK/ERK1/2	Positive association $P < 0.01$	Bergqvist et al. (2006)
P-S118	P-MAPK/ERK1/2	Positive association $P < 0.0005$	Jiang et al. (2007) [4]
P-S118	P-MAPK/ERK1/2	Positive association $P < 0.0001$	Yamashita et al. (2008) [5]
P-S118	P-p90RSK	Positive association $P < 0.0005$	Jiang et al. (2007) [4]
P-S118	P-AKT	Positive association $P = 0.035$	Jiang et al. (2007) [4]
P-S118	P-AKT	Positive association $P < 0.0001$	Yamashita et al. (2008) [5]
P-S167	P-MAPK/ERK1/2	Positive association $P < 0.0005$	Jiang et al. (2007) [4]
P-S167	P-MAPK/ERK1/2	Positive association $P < 0.0001$	Yamashita et al. (2008) [5]
P-S167	P-AKT	Positive association $P = 0.005$	Jiang et al. (2007) [4]
P-S167	P-AKT	Positive association $P < 0.0001$	Yamashita et al. (2008) [5]
P-S167	P-p90RSK	Positive association $P < 0.001$	Jiang et al. (2007) [4]

HER2+ and HER2− tumors were matched for equivalent ER and PR expression as well as grade and tumor type, found no significant differences in the frequency or expression levels of P-S118 [22]. As well, using this latter cohort we found no significant difference in terms of P-S167 positivity between ER+/HER2+ tumors versus ER+/HER2− tumors (Fishers exact test $P = 0.43$). Although in this study there was a trend towards decreased P-S167 expression in HER2 over-expressing tumors compared to non-expressing tumors (median IHC score HER2 over-expressors = 50 vs. median for non-expressors = 67.5, $P = 0.10$, Mann Whitney two tailed) this was not statistically significant (unpublished data). These data confirm the lack of association of P-S167 with HER2 over-expression, found by Jiang et al. [19]. Therefore the majority of data, so far published, are consistent with the conclusion that increased detection of P-S118 and/or P-S167 expression is not associated with HER2 over-expression in primary breast tumors that are ERα positive *in vivo*.

5. Multiple phosphorylated forms of ERα

Another common theme emerging from the results summarized in Table 2 is that multiple phosphorylated forms of ERα can be found in any one tumor sample [10,19,25]. Our recent results [10] have extended this further to measure up to seven different P-ERα forms in over 300 ER+ breast tumor samples. The phosphorylated forms of ERα that we validated and measured by IHC were P-S104/106; P-S118, P-S167, P-S282, P-S294, P-T311 and P-S559 [10]. We found that any one P-ERα isoform was positively correlated with one or more other P-ERα isoforms. For example, P-S167 was positively correlated with P-S118 (Spearman $r = 0.46$, $P < 0.0001$, $n = 360$). To investigate if the same ER molecules were phosphorylated on multiple sites or whether there were distinct separate molecular populations of each P-ERα versus populations of ERα phosphorylated at multiple sites, we immunoprecipitated (IP) ER phosphorylated at S118 with a specific antibody [22] from estrogen treated MCF7 cell extracts followed by Western blotting using an antibody specific to P-S167. Fig. 3 illustrates that IP (beads) with an antibody specific to P-S118 ERα, which quantitatively removes signal from the extract (see decrease P-Ser118 signal in supernantant), co-IP ERα molecules also phosphorylated at S167 identified by Western blotting of the beads extract with an antibody specific for P-S167. This suggests that there is a population of ER molecules phosphorylated at both S118 and S167, supporting the idea that there is a population of ERα molecules phosphorylated at multiple sites.

The presence of multiple types of phosphorylated ERα isoforms in any one tumor sample may reflect simply the fact that in most model systems, estrogen treatment increases levels of ERα phosphorylated at most of the sites measured [5,9,12,22], therefore it is possible that all or any phospho-ERα represent the presence of an intact, estrogen dependent ER signaling pathway in primary human breast tumors. This conclusion is consistent with the association of most phosphorylated ERα's often with increased PR expression. Alternatively, it is possible that the phosphorylation of ERα at any one site may induce a conformational change which increases the likelihood of phosphorylation at the other sites [27].

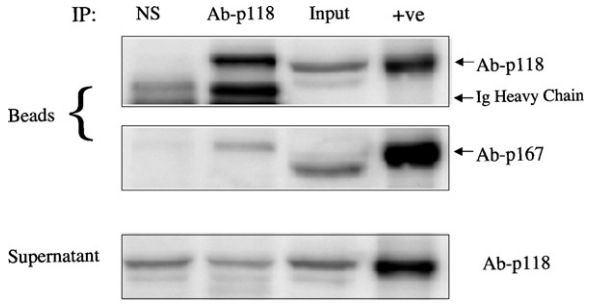

Fig. 3. MCF-7 cells were seeded in 15 cm dishes (10^6 cells/dish). Twenty-four hours later medium was changed to medium containing 2× charcoal-treated serum for 5 days (media changed every second day) and then treated with estradiol (10 nM, E_2) for 30 min. Cell extracts were prepared, immunoprecipitated (IP) with anti-P-S118 antibody (mouse monoclonal) or an irrelevant isotype matched anti-insulin receptor antibody (NS, Santa Cruz). Western blotting was either with anti-P-S118 antibody (mouse monoclonal, Cell Signaling) or anti-P-S167 (rabbit polyclonal, Upstate). Input represents 5% of total cell extract subjected to IP, and positive control is 50 μg of cell extract protein from E_2 treated COS1 cells transfected with ERα expression plasmid. The IP proteins = beads. Aliquots of the supernatant after IP were also Western blotted = supernatant.

6. Kinases potentially involved in phosphorylation of ERα in human breast cancer *in vivo*

Several kinases have been implicated in mediating the phosphorylation of ERα at various sites [5]. However, the kinases, that are involved in breast cancer *in vivo* are not known. To gain some insight into this matter, some of the studies, listed in Tables 1 and 2, have also determined the relationship of individual P-ERα expression to active forms of some of the kinases, implicated in cell line model systems [5,12], in human breast tumor samples [15,17,19,25]. Statistically significant positive associations of P-S118 and/or P-S167 have been found with several activated kinases as shown in Table 3. The data suggests that activated forms of MAPK/ERK1/2, p90RSK and/or P-AKT may be involved in phosphorylation of ERα in primary human breast tumors *in vivo*, under conditions where either P-S118 or P-S167 is associated with an intact estrogen dependent signaling pathway and better outcome to endocrine therapy.

7. Summary and conclusions

Multiple phosphorylated forms of ERα can be detected in multiple human breast tumor biopsy samples, thus establishing the

relevance of investigating the regulation and function of ERα phosphorylation to human breast cancer. Although details may differ, generally phosphorylated forms of ERα in primary human breast tumors are associated with other known markers of an intact estrogen dependent ER signaling pathway and are associated with a better clinical outcome on tamoxifen therapy and not *de novo* resistance to tamoxifen. These data raise the possibility that expanding ER status to incorporate post-translational profiling, such as the phosphorylation status of ERα, may be useful in more precisely selecting subgroups of patients that benefit best from endocrine therapy. As well, identifying the important kinases involved in phosphorylating ERα in human breast tumors *in vivo* may provide targets for new endocrine therapies in breast cancer patients.

References

[1] J. Harvey, G. Clark, C. Osborne, D. Allred, Estrogen receptor status by immunohistochemistry is superior to the ligand binding assay for predicting response to adjuvant endocrine therapy in breast cancer, J. Clin. Oncol. 17 (5) (1999) 1474–1481.

[2] C. Osborne, Steroid hormone receptors in breast cancer management, Breast Cancer Res. Treat. 51 (1998) 227–238.

[3] C. Osborne, Tamoxifen in the treatment of breast cancer, N. Engl. J. Med. 339 (1998) 1609–1618.

[4] C. Osborne, R. Schiff, Estrogen-receptor biology: continuing progress and therapeutic implications, J. Clin. Oncol. 23 (2005) 1616–1622.

[5] D. Lannigan, Estrogen receptor phosphorylation, Steroids 68 (1) (2003) 1–9.

[6] N. Weigel, N. Moore, Steroid receptor phopshorylation: a key modulator of multiple receptor functions, Mol. Endocrinol. 21 (2007) 2311–2319.

[7] L. Murphy, P. Watson, Is oestrogen receptor-beta a predictor of endocrine therapy responsiveness in human breast cancer? Endocr. Relat. Cancer 13 (2006) 327–334.

[8] M. Al-Dhaheri, B. Rowan, Application of phosphorylation site-specific antibodies to measure nuclear receptor signaling: characterization of novel phosphoantibodies for estrogen receptor alpha, Nucl. Recept. Signal. 4 (2006) e007 (Epub April 28).

[9] C. Williams, C.L. Smith, B.G. Rowan, Identification of four novel phosphorylation sites in estrogen receptor α: impact on receptor-dependent gene expression and phosphorylation by protein kinase CK2, in: The Endocrine Society's 89th Annual Meeting, Toronto, Canada, 2007, pp. P2–P258.

[10] G. Skliris, B. Rowan, M. Al-Dhaheri, C. Williams, S. Troup, S. Begic, M. Parisien, P. Watson, L. Murphy, Immunohistochemical validation of multiple phospho-specific epitopes for estrogen receptor α (ERα) in tissue microarrays (TMA) of ERα positive human breast carcinomas, Breast Cancer Res. Treat., 2008 Dec 23. [Epub ahead of print].

[11] C. Atsriku, D. Britton, J. Held, B. Schilling, G. Scott, B. Gibson, C. Benz, M. Baldwin, Systematic mapping of posttranslational modifications in human estrogen receptor alpha, with emphasis on novel phosphorylation sites, Mol. Cell. Proteomics (November (3)) (2008) (Epub ahead of print).

[12] L. Murphy, G. Weitsman, G. Skliris, E. Teh, L. Li, B. Peng, J. Davie, K. Ung, Y. Niu, S. Troup, et al., Potential role of estrogen receptor alpha phosphorylation at serine 118 in human breast cancer in vivo, J. Steroid Biochem. Mol. Biol. 102 (2006) 139–146.

[13] R. Schiff, S. Massarwah, J. Shou, L. Bharwani, G. Arpino, M. Rimawi, C. Osborne, Advanced concepts in estrogen receptor biology and breast cancer endocrine resistance: implicated role of growth factor signaling and estrogen receptor coregulators, Cancer Chemother. Pharmacol. 56 (Suppl. 1) (2005) s10–s20.

[14] L. Murphy, Y. Niu, L. Snell, P. Watson, Phospho-serine-118 estrogen receptor-alpha expression in primary human breast tumors in vivo is associated with better disease outcome in women treated with tamoxifen, Clin. Cancer Res. 10 (17) (2004) 5902–5906.

[15] L.C. Murphy, T. Cherlet, A. Adeyinka, Y. Niu, L. Snell, P. Watson, Phospho-serine-118 estrogen receptor-alpha detection in human breast tumors in vivo, Clin. Cancer Res. 10 (2004) 1354–1359.

[16] J. Gee, J. Robertson, E. Gutteridge, I. Ellis, S. Pinder, M. Rubini, R. Nicholson, Epidermal growth factor receptor/HER2/insulin-like growth factor receptor signalling and oestrogen receptor activity in clinical breast cancer, Endocr. Relat. Cancer 12 (Suppl. 1) (2005) S99–S111.

[17] N. Sarwar, J. Kim, J. Jiang, D. Peston, H. Sinnett, P. Madden, J. Gee, R. Nicholson, A. Lykkesfeldt, S. Shousha, et al., Phosphorylation of ERalpha at serine 118 in primary breast cancer and in tamoxifen-resistant tumours is indicative of a complex role for ERalpha phosphorylation in breast cancer progression, Endocr. Relat. Cancer 13 (2006) 851–861.

[18] J. Bergqvist, G. Elmberger, J. Ohd, B. Linderholm, J. Bjohle, H. Hellborg, H. Nordgren, A. Borg, L. Skoog, J. Bergh, Activated ERK1/2 and phosphorylated oestrogen receptor alpha are associated with improved breast cancer survival in women treated with tamoxifen, Eur. J. Cancer 42 (2006) 1104–1112.

[19] J. Jiang, N. Sarwar, D. Peston, E. Kulinskaya, S. Shousha, R. Coombes, S. Ali, Phosphorylation of estrogen receptor-alpha at Ser167 is indicative of longer disease-free and overall survival in breast cancer patients, Clin. Cancer Res. 13 (2007) 5769–5776.

[20] C. Holm, M. Kok, R. Michalides, R. Fles, R. Koornstra, J. Wesseling, M. Hauptmann, J. Neefjes, J. Peterse, O. Stål, et al., Phosphorylation of the oestrogen receptor alpha at serine 305 and prediction of tamoxifen resistance in breast cancer, J. Pathol. (September (23)) (2008) (Epub ahead of print).

[21] D. Chen, E. Washbrook, N. Sarwar, G. Bates, P. Pace, V. Thirunuvakkarasu, J. Taylor, R. Epstein, F. Fuller-Pace, J. Egly, et al., Phosphorylation of human estrogen receptor alpha at serine 118 by two distinct signal transduction pathways revealed by phosphorylation-specific antisera, Oncogene 21 (32) (2002) 4921–4931.

[22] G. Weitsman, L. Li, G. Skliris, J. Davie, K. Ung, L. Curtis-Snell, L. Tomes, P. Watson, L. Murphy, Estrogen receptor-alpha phosphorylated at serine 118 is present at the promoters of estrogen-regulated genes and is not altered due to Her2 overexpression, Cancer Res. 66 (2006) 10162–10170.

[23] R. Barnes, M. Parisien, L. Murphy, P. Watson, Influence of evolution in tumor biobanking on the interpretation of ranslational research, Cancer Epidemiol. Biomarkers Prev. 17 (12) (2008) 3344–3350.

[24] H. Yamashita, M. Nishio, S. Kobayashi, Y. Ando, H. Sugiura, Z. Zhang, M. Hamaguchi, K. Mita, Y. Fujii, H. Iwase, Phosphorylation of estrogen receptor alpha serine 167 is predictive of response to endocrine therapy and increases postrelapse survival in metastatic breast cancer, Breast Cancer Res. 7 (2005) R753–R764.

[25] H. Yamashita, M. Nishio, T. Toyama, H. Sugiura, N. Kondo, S. Kobayashi, Y. Fuji, H. Iwase, Low phosphorylation of estrogen receptor {alpha} (ER{alpha}) serine 118 and high phosphorylation of ER{alpha} serine 167 improve survival in ER-positive breast cancer, Endocr. Relat. Cancer 15 (2008) 755–763.

[26] M. Dowsett, S. Johnston, L.A. Martin, J. Salter, M. Hills, S. Detre, M.C. Gutierrez, S.K. Mohsin, J. Shou, D.C. Allred, R. Schiff, C.K. Osborne, I. Smith, Growth factor signalling and response to endocrine therapy: the Royal Marsden Experience, Endocr. Relat. Cancer 12 (Suppl. 1) (2005) S113–S117.

[27] C. Yang, H. Xin, J. Kelley, A. Spencer, D. Brautigan, B. Paschal, Ligand binding to the androgen receptor induces conformational changes that regulate phosphatase interactions, Mol. Cell. Biol. 27 (2007) 3390–3404.