Breast Cancer Online, **10**(7), Page 1 of 3, ***e13***
doi:10.1017/S1470903107006578

ISSN 1470-9031

Breast Cancer Online
www.bco.org

Journals Club

Review of: Estrogen receptor β expression is associated with tamoxifen response in ERα-negative breast carcinoma

L. Murphy

Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada.

Citation of original article:
S. K. Gruvberger-Saal, P.-O. Bendahl, L. H. Saal, M. Laakso, C. Hegardt, P. Edén, C. Peterson, P. Malmström, J. Isola, A. Borg, M. Fernö. ***Clinical Cancer Research*** 2007; **13**: 1987–1994.

Abstract of the original article:
Purpose: Endocrine therapies, such as tamoxifen, are commonly given to most patients with estrogen receptor (ERα)-positive breast carcinoma but are not indicated for persons with ERα-negative cancer. The factors responsible for response to tamoxifen in 5% to 10% of patients with ERα-negative tumors are not clear. The aim of the present study was to elucidate the biology and prognostic role of the second ER, ERβ, in patients treated with adjuvant tamoxifen. **Experimental design**: We investigated ERβ by immunohistochemistry in 353 stage II primary breast tumors from patients treated with 2 years adjuvant tamoxifen, and generated gene expression profiles for a representative subset of 88 tumors. **Results**: ERβ was associated with increased survival (distant disease-free survival, $P = 0.01$; overall survival, $P = 0.22$), and in particular within ERα-negative patients ($P = 0.003$; $P = 0.04$), but not in the ERα-positive subgroup ($P = 0.49$; $P = 0.88$). Lack of ERβ conferred early relapse (hazards ratio, 14; 95% confidence interval, 1.8–106; $P = 0.01$) within the ERα-negative subgroup even after adjustment for other markers. ERα was an independent marker only within the ERβ-negative tumors (hazards ratio, 0.44; 95% confidence interval, 0.21–0.89; $P = 0.02$). An ERβ gene expression profile was identified and was markedly different from the ERα signature. **Conclusion**: Expression of ERβ is an independent marker for favorable prognosis after adjuvant tamoxifen treatment in ERα-negative breast cancer patients and involves a gene expression program distinct from ERα. These results may be highly clinically significant, because in the United States alone, approximately 10 000 women are diagnosed annually with ERα-negative/ERβ-positive breast carcinoma and may benefit from adjuvant tamoxifen.

Review

For many years it has been appreciated that patients whose breast tumors are ERα negative in general do not benefit from tamoxifen or other endocrine therapies. However, multiple studies have reported the presence of a small cohort of patients whose tumors are ERα negative but do respond to tamoxifen therapy [1]. The size of this cohort has been estimated as being less than 10% of patients with ERα-negative tumors. The reasons for an effect of tamoxifen in ERα-negative tumors have been unclear although some suggestions include false negative assays due to technical issues, or tamoxifen effects via an ER-independent mechanism(s) [2]. Results presented in this paper

Correspondence to: Leigh Murphy, Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada R3E 0V9. E-mail: lcmurph@cc.umanitoba.ca

Received: 31/07/07
Accepted: 14/07/07
BCO/657/2007/JC

suggest that tamoxifen may have beneficial effects in ERα-negative but ERβ-expressing breast cancer.

The role of ERβ in human breast cancer is unclear. Previous studies aimed at gaining insight into the role of ERβ in breast cancer by determining associations of ERβ with clinical–pathological markers and responsiveness to adjuvant tamoxifen therapy, predominantly studied patients whose breast tumors were ERα positive [3]. Although a small number of patients in the previously investigated cohorts had ERα-negative tumors, the numbers were likely never high enough to allow stratification by ERα status. Furthermore, adequate evidence is now available that 15–17% of primary breast tumors are ERα negative but express detectable levels of ERβ-like proteins [4]. The current study however was able to investigate 353 patients with stage II breast cancer who had been treated uniformly with 2 years of tamoxifen monotherapy without selection for ER status. Therefore, the cohort consisted of the usual unselected distribution of ER-positive and ER-negative breast tumors, i.e. 70% ER positive and 30% ER negative. This patient cohort was selected from two earlier trials of tamoxifen monotherapy: (1) one that compared 2- and 5-year tamoxifen treatment duration in postmenopausal women with stage II disease [5] and (2) one that compared 2 years of tamoxifen treatment with untreated premenopausal women with stage II disease [6]. The specific aims of the study were (1) to investigate ERβ protein levels as a predictor of therapy response in both ERα-positive and ERα-negative breast cancer patients, uniformly treated for 2 years with adjuvant tamoxifen; (2) to identify a gene expression signature for ERβ status compared with an ERα-associated expression signature.

ERβ expression was determined immunohistochemically (IHC) using a cocktail of 14C8 (total ERβ-like) and PPG5/10 (ERβ1) monoclonal antibodies. Both these antibodies have been extensively validated and used previously by multiple laboratories to determine ERβ-like proteins by IHC in breast cancer [7]. While the rationale for using the mix was not given, all known ERβ isoforms would be detected using this cocktail and no discrimination among isoforms can be made. The results therefore have to be interpreted in the context of total ERβ-like protein determination. However, this distinction and its likely impact on the interpretation of the data are not discussed. ERβ negativity was defined as no to weak staining (over background) in $<$20% of carcinoma cells. Whether nuclear or cytoplasmic staining or both were scored was not stated. Gene expression profiling of a representative set of 88 breast tumors was undertaken using cDNA microarrays with 27 648 spots produced in the SWEGENE Microarray Facility, Department of Oncology, Lund University.

Key findings:

(1) In the whole cohort, ERβ was significantly associated with disease-free survival ($P = 0.01$) with a trend to association with overall survival ($P = 0.22$). In subgroup analysis stratified by ERα status, ERβ was significantly associated with disease-free survival ($P = 0.003$) and overall survival ($P = 0.04$) only in the ERα-negative group.
(2) ERα was only an independent marker of better disease-free survival in the ERβ-negative group.
(3) An ERβ gene expression profile was identified, which was different from the ERα gene expression signature.

The implications of this article are potentially exciting. ERα-negative breast cancers usually have a more aggressive biology and treatments for patients with ERα-negative breast cancer are usually confined to the more toxic chemotherapies. The precise identification of a subgroup within this cohort that would benefit from less-toxic endocrine therapies would be a significant benefit to breast cancer patients. However, there are several issues in this study that raise many questions.

Why only the 2 years of tamoxifen treatment group was used in the current study but not the 5-year group is not explained. Especially since the maximum benefit from tamoxifen therapy has been shown multiple times to require 5 years of tamoxifen therapy [8]. Maybe this is why no significant benefit of tamoxifen therapy is seen in the ERα-positive cohort as a whole in this study, when multiple other studies and meta-analyses of the studies have clearly established the predictive role of ERα status in endocrine therapy response. A similar analysis to the one published in this article on the 5-year tamoxifen-treated cohort [5] would be an interesting comparison.

Another result from the current study, which stands out as different compared to those previously published in the literature, is the finding that ERα positivity was associated with a greater number of lymph nodes with metastases ($P = 0.006$). Such a relationship has not been found previously in much larger studies [9]. Perhaps this indicates a bias within the cohort studied in the above paper. As well the ERα-negative PR-positive category is 10% in the current study, which is somewhat high compared to other studies and may indicate a cohort bias or different cut-off points for defining ERα positivity.

With respect to the ERβ results obtained in the current study, the percent defined as ERβ positive is similar to those of other published studies [3,4]. But it must be emphasized that there are no standards or clinically relevant cut-off values associated with the definition of ERβ positivity or negativity and the rationale for the cut-off used in this study is not given.

https://doi.org/10.1017/S1470903107006578 Published online by Cambridge University Press

An interesting finding in this study is the association of increased ERβ expression with high percent of S-phase fraction (SPF). Generally, high SPF is associated with poorer clinical outcome [10], but in this case despite the association of ERβ with high SPF, higher ERβ is associated with better clinical outcome, which seems counter-intuitive and needs discussion. However, the positive association of ERβ with SPF is consistent with the positive association of ERβ with the proliferation marker Ki67 in ERα-negative breast tumors, found in several studies to date [11]. The meaning of this is unclear since increased expression of ERβ1 in cancer cells in culture generally inhibits proliferation and cell cycling [12,13]. With regard to this issue, since total ERβ-like proteins are measured, it is unclear what the predominant ERβ isoform is in the tumors in this study or in breast tumors in vivo generally, or if the relative expression of ERβ isoforms at the protein level changes between ERα-positive and ERα-negative tumors [14].

Overall, the current study is different from the majority of other published studies where an association of higher ERβ-like protein expression with better clinical outcome with tamoxifen in general is found in breast cancer cohorts that are exclusively or predominantly ERα positive [3], and where ERα-positive status is the major predictor of treatment response to tamoxifen [1]. These apparent discrepancies require discussion.

This study is the first to identify an ERβ gene expression profile in human breast tumors, and not surprisingly [14] it is distinct from the ERα gene expression signature. However, the lack of validation of any candidate ERβ-associated gene markers in breast tumors identified in this study using other approaches, together with the lack of discussion of any common (or lack thereof) ERβ-associated gene markers found in other systems [15,16] leaves the reader with little insight into the potential value of this expression profile. In addition, identification of differences between gene expression profiles for ERβ-positive vs. ERβ-negative tumors that are also ERα negative, if any, would have been relevant to the findings of the current study.

However exciting this study is, it requires replication in other cohorts by other groups retrospectively, as well as prospectively.

References

1. EBCTCG. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998; **354**: 1451–1467.
2. Eisen S, Brown H. Selective estrogen receptor (ER) modulators differentially regulate phospholipase D catalytic activity in ER-negative breast cancer cells. *Mol Pharmacol* 2002; **62**: 911–920.
3. Murphy L, Watson P. Is oestrogen receptor-beta a predictor of endocrine therapy responsiveness in human breast cancer? *Mol Pharmacol* 2006; **13**: 327–334.
4. Murphy L, Cherlet T, Lewis A, Banu Y, Watson P. New insights into estrogen receptor function in human breast cancer. *Ann Med* 2003; **35**(8): 614–631.
5. Swedish Breast Cancer Cooperative Group. Randomized trial of two versus five years of adjuvant tamoxifen for postmenopausal early stage breast cancer. *J Natl Cancer Inst* 1996; **88**: 1543–1549.
6. Ryden L, Jonsson P, Chebil G, *et al*. Two years of adjuvant tamoxifen in premenopausal patients with breast cancer: a randomised, controlled trial with long-term follow-up. *Eur J Cancer* 2005; **41**: 256–264.
7. Weitsman G, Skliris G, Ung K, *et al*. Assessment of multiple different oestrogen receptor β antibodies for their ability to immunoprecipitate under chromatin immunoprecipitation conditions. *Breast Cancer Res Treat* 2006; **100**: 23–31.
8. Fisher B, Dignam J, Bryant J, Wolmark N. Five versus more than five years of tamoxifen for lymph node-negative breast cancer: updated findings from the National Surgical Adjuvant Breast and Bowel Project B-14 randomized trial. *J Natl Cancer Inst* 2001; **93**: 684–690.
9. Clark G, Osborne C, McGuire W. Correlations between estrogen receptor, progesterone receptor and patient characteristics in human breast cancer. *J Clin Oncol* 1984; **2**: 1102–1108.
10. Wenger C, Clark G. S-phase fraction and breast cancer – a decade of experience. *Breast Cancer Res Treat* 1998; **51**: 255–265.
11. Skliris G, Leygue E, Curtis-Snell L, Watson P, Murphy L. Expression of oestrogen receptor-beta in oestrogen receptor-alpha negative human breast tumours. *Br J Cancer* 2006; **95**: 616–626.
12. Paruthiyil S, Parmar H, Kerekatte V, Cunha G, Firestone G, Leitman D. Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Res* 2004; **64**: 423–428.
13. Strom A, Hartman J, Foster J, Kietz S, Wimalasena J, Gustafsson JA. Estrogen receptor β inhibits 17β-estradiol-stimulated proliferation of the breast cancer cell line T-47D. *Proc Natl Acad Sci USA* 2004; **101**(6): 1566–1571.
14. Skliris G, leygue E, Watson P, Murphy L. Estrogen receptor alpha negative breast cancer patients: estrogen receptor beta as a therapeutic target. *J Steroid Biochem Mol Biol* **2007**: in press.
15. Monroe D, Getz B, Johnsen S, Riggs BL, Khosla S, Spelsberg T. Estrogen receptor isoform specific regulation of endogenous gene expression in human osteoblastic cell lines expressing either ERα or ERβ. *J Cell Biochem* 2003; **90**: 315–326.
16. Monroe D, Secreto F, Subramaniam M, Getz B, Khosla S, Spelsberg T. Estrogen receptor alpha and beta heterodimers exert unique effects on estrogen- and tamoxifen-dependent gene expression in human U2OS osteosarcoma cells. *Mol Endocrinol* 2005; **19**: 1555–1568.

https://doi.org/10.1017/S1470903107006578 Published online by Cambridge University Press