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A Simple Method to Assess Estrogen Receptor Gene (ESR1) Amplification in Paired Biopsies from Primary Tumor and Recurrence in Breast Cancer Patients Receiving Endocrine Therapy

Cristóbal Maiz[1], David Oddó[2], Francisca Alfaro[2], Alejandra Villarroel[2], Francisco Acevedo[3], Alejandra Pérez Sepulveda[3], Sabrina Muñiz RN[4], Fernando Silva[3], Andrés Valdivia[3], Tomas Merino[3], Mauricio P Pinto[3] and César Sánchez[3]*

[1]*Department of Surgical Oncology, Faculty of Medicine, Pontifical Catholic University of Chile, Chile 2Pathological Anatomy Laboratory, Faculty of Medicine, Pontifical Catholic University of Chile, Chile*

[3]*Department of Hematology Oncology, Faculty of Medicine, Pontifical Catholic University of Chile, Chile*

[4]*Hematology-Oncology Unit, Dr Sótero del Río Healthcare Complex, Chile*

***Corresponding author:** César Sánchez, Department of Hematology-Oncology; Faculty of Medicine Pontifical Catholic University of Chile, Chile

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Abstract

Globally, Breast Cancer (BC) is the leading cause of cancer death among women. About 75% of patients are diagnosed with hormone-dependent tumors and are set to receive Endocrine Therapy (ET) targeting the estrogen receptor. Unfortunately, a significant proportion of these patients develops ET resistance. Still controversial, studies have proposed that Estrogen Receptor-Alpha Gene (ESR1) alterations may underlie ET resistance. Here, we describe the use of a Chromogenic in Situ Hybridization (CISH) assay for the assessment of ESR1 amplification in primary tumors and recurrences. This assay could be a useful clinical tool with therapeutic implications for estrogen receptor positive BC patients.

Keywords: ESR1; Breast Cancer; Estrogen-Receptor Amplification; Endocrine Therapy; Endocrine Therapy Resistance

Abbreviations: ET: Endocrine Therapy; CISH: Chromogenic in Situ Hybridization; BC: Breast Cancer; FFPE: Formalin-Fixed Paraffin Embedded; HER-2: Human Epidermal Growth Factor Receptor Type-2; ISH: In Situ Hybridization

Introduction

Worldwide, Breast Cancer (BC) remains as the leading cause of cancer-related death for women [1]. Approximately a 75% of breast tumors stain positive for the Estrogen Receptor (ER+). Upon diagnosis, ER+ BC patients are candidates to Endocrine Therapy (ET). Indeed, positivity for ER in tumors is a predictor for ET responsiveness [2,3]. Also, tumoral ER expression has prognostic implications, ER+ BC patients that receive ET display better survival rates versus ER- counterparts [4-6]. Furthermore, in early stage ER+ BC patients ET reduces mortality by 50% [4]. However, almost 25% of these patients will eventually develop ET resistance. Among metastatic ER+ BC patients, this % is much higher. Clinically, ET resistance is manifested as recurrent disease during or after adjuvant treatment [3]. For these patients, metastasis is the main cause of death [7]. Studies have postulated a variety of ET resis-

tance mechanisms, however one of the best described to date is ER-alpha gene (ESR1) amplification [8]. In fact, ESR1 amplification has been described in both primary tumors and metastases [9–11]. Currently, the assessment of gene amplification is a standard procedure routinely performed in pathology laboratories.

For example, Human Epidermal Growth Factor Receptor Type-2 (HER2) gene amplification is routinely determined by In Situ Hybridization (ISH) using Formalin-Fixed Paraffin Embedded (FFPE) tissue from mammary neoplasms for BC diagnosis. Herein, we report ESR1 amplification in paired biopsies (primary tumor and recurrence) from BC patients that developed ET resistance. To the best of our knowledge, this is the first report on this clinically relevant method using paired samples from primary tumor and its recurrence.

Materials and Methods

Study Design and Ethics Approval

This study is an observational, retrospective patient series. Research was approved by ethics committee at the School of Medicine in the Pontificia Universidad Catolica de Chile (approval #190605010, dated on August 1st, 2019).

Patients, Samples and Clinical Data

Patients with ER+ BC at diagnosis who were treated with ET and recurred during or after finishing treatment were selected from our database. Stage IV patients were excluded. FFPE tissue of tumors were obtained from a biobank. Anonymized demographics and clinical data were obtained from institutional database.

ESR1 Gene Amplification Assessment by Chromogenic in Situ Hybridization

FFPE blocks were sectioned at 5 um. After deparaffinization and hydration, a commercial ZytoLight® SPEC ESR1/CEN 6 Dual Color Probe (cat# Z-2069-200; ZytoVision GmbH, Germany) was used, following manufacturer's instructions. After ISH procedure, samples were blindly evaluated by an expert pathologist. ESR1 amplification was defined as a ratio of ESR1 to centromere at ≥4.

Statistical Analysis

Descriptive statistical analysis was used. Parametric or nonparametric tests were used when appropriate. Also, Spearman or Pearson correlations were assessed. Statistical significance was set at $p<0.05$. For data analysis, STATA v.14 and GraphPad Prism v7.0. software were used.

Results

Patients' basic characteristics are summarized in Table 1. Median age was 50.5 years (range: 37-73). Histologically, 85% of patient samples were classified as ductal carcinoma, and a 64.28% were treated with tamoxifen prior to ET resistance. Median recurrence free survival was 36 months (range: 11-219). A total of 14 paired biopsies (primary tumor and recurrence) from ER+ BC were analyzed. Figure 1 shows two invasive ductal carcinomas with or without ESR1 amplification in upper and lower panels, respectively. Left panels show hematoxylin & eosin stains. Right panels show ESR1/CEN6 stains. Note the presence of several positive nuclei in the upper right panel. Overall, a 23% of primary tumors (3/10) were ESR1-amplification+, whereas in recurrence was 50% (4/8). Also, in 3 out of 7 cases (42.9%) we observed discrepancies in ESR1-amplification status between the primary tumor and the recurrence; Within this subset, 2 went from ESR1-amplification- to + (primary versus recurrence), and 1 recurrence lost the ESR1-amplification observed in the primary tumor. Additionally, patients with recurrent disease and ESR1-amplification+ tended to be older (63 versus 51 years, respectively), displayed higher BMI index and ER expression. They also had shorter disease-free survival rates against patients that changed their status from ESR1-amplification+ to - status. Unfortunately, all these differences were not statistically significant.

Table 1: Patients' basic characteristics by ESR1-amplification status.

	ESR1 status on primary tumor		*ESR1* status recurrence on biopsy		
Characteristics	**Not Amplified (n=10)**	**Amplified (n=3)**	**Not Amplified (n=4)**	**Amplified (n=4)**	**p-value**
Age at diagnosis (years)	50.5 (37-73)	50 (45-61)	47 (37-52)	63.5 (61-73)	0.14
BMI (m/Kg^2)	25 ± 4.27	27 ± 1.83	28.1 ± 5.20	30.6 ± 4.70	0.29
Tumor size (cm)	5.72 ± 2.71	1.05 ± 0.35	5.1 ± 3.80	1.95 ± 0.07	0.14
Nº compromised lymph nodes	3.5 (1-15)	0	1 (1-3)	3 (2-4)	0.053
% Compromised lymph nodes	21.75 ± 13.28	0	13.68 ± 17.45	63.33 ± 51.85	0.079
% ER expresion	79.88 ± 13.68	73.0 ± 37.51	90.5 ± 11.09	91.75 ± 8.80	0.50
% PR expression	46.25 ± 40.95	60.0 ± 36.06	58.33 ± 38.84	26.67 ± 37.86	0.50
Time to recurrence (months)	45 (16-219)	33 (11-35)	62,5 (24-147)	28.5 (11-219)	0.46

*ESR1: Estrogen Receptor-Alpha Gene; BMI: Body Mass Index; ER: Estrogen Receptor; PR: Progesterone Receptor.

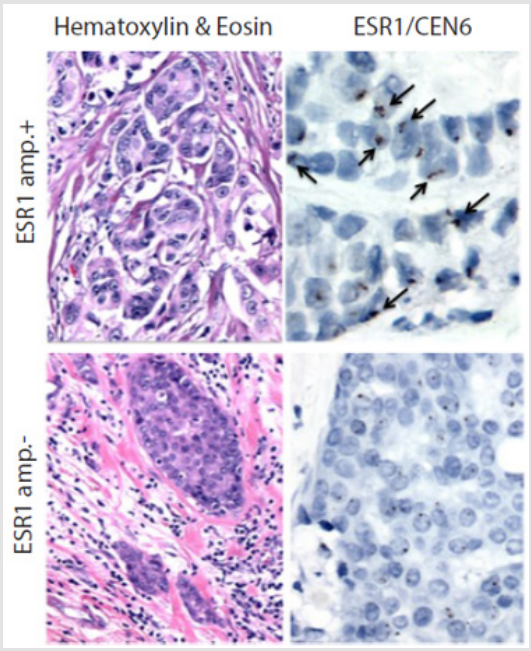

Figure 1: Analysis of ESR1 amplification status on invasive ductal carcinoma biopsies. Left panels show Hematoxylin & Eosin stains. Right panels show Chromogenic in Situ Hybridization (CISH) stains against ESR1/CEN6. Upper panel shows a patient ESR1-amplification+ (ESR1-amp. +), arrows indicate positive nuclei (upper right). Lower panel shows a patient ESR1-amplification-. Magnification: 1,000x.

Discussion

To the best of our knowledge, this is the first report on ESR1-amplification by CISH on paired samples from primary tumors and metastases obtained from ER+ BC patients. As reported previously, our data suggest ESR1 amplification is more frequently observed in recurrent tumors. Previous studies in early ER+BC patients demonstrate ESR1 genomic alterations are rare, ranging from 2-5% [12-14], suggesting they are not related to carcinogenesis [15]. In contrast, ESR1 alterations are far more frequent among metastatic ER+ BCs, ranging from 20-55% [16,17], suggesting a role in metastasis. Massive sequencing technologies and the development of collaborative efforts like The Cancer Genome Atlas (TCGA) have helped to elucidate the genomic complexity of BC. These studies have demonstrated that copy number alterations are commonly seen in the ESR1 gene. Furthermore, ESR1 amplifications are related to increases in functional ER protein. Concomitantly, several point mutations can affect the ER-Ligand Binding Domain (LBD), generating a ligand-independent constitutively active-ER [18]. Mutant ER variants may play a role in ET resistance in patients and may confer some advantages to expressing cancer cells, favoring proliferation in estrogen-deprived conditions or even in the presence of ET drugs such as tamoxifen or fulvestrant [19,20]. Indeed, studies demonstrate that both tamoxifen and fulvestrant can inhibit the activity of wild-type and mutant-ERs, however inhibition of mutant ER requires higher doses, suggesting that mutant receptors play a role in ET resistance [19,20].

Previously, studies have demonstrated that the rate of ESR1 amplification in BC is about 20% in both primary tumors and metastases [9–11]. Therefore, ET resistance cannot be fully attributed to this phenomenon. However, the amplification of mutant ESR1 variants may play a role in ET resistance. Interestingly, we found that 3 out of 10 (42.9%) displayed ESR1 amplification in primary tumors and 4 out of 8 (50%) had it at recurrence. Evidently, our study has certain limitations including the number of cases analyzed, also we did not assess point mutations on ESR1. Future studies should confirm our findings and elucidate the role of ESR1 amplification on the development of ET resistance.

Acknowledgement

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Conflicts of Interest

The authors declare no conflicts of interest.

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César Sánchez. Biomed J Sci & Tech Res