Comparison of fluorescence in situ hybridization of estrogen receptor genetic locus with protein expression in invasive breast carcinoma

Julie Ann Mayer[1], Tam Pham[1], Karina Wong[1], Anthony Lucci[2], Farideh Z. Bischoff[1] and Savitri Krishnamurthy[3]

[1]Biocept, Inc., San Diego CA; [2]Department of Surgical Oncology, [3]Department of Pathology, University of Texas MD Anderson Cancer Center, Houston TX

THE UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER

ABSTRACT

INTRODUCTION: Evaluation of estrogen receptor (ESR1) status is recommended in all breast cancer patients and is generally performed using immunohistochemistry (IHC). However, the analytical sensitivity of IHC in detecting low levels of ESR1 amplification is often poor and likely due to methodological variation. Though FISH (fluorescent *in-situ* hybridization) has been proposed as an alternative approach for detection of ESR1 gain, results have been controversial with few studies evaluating concordance between FISH and IHC. In this study, we describe the performance of FISH using an ESR1 probe and correlation of the results with IHC for ESR protein expression.

METHODS: FISH and IHC for ESR1 was performed on adjacent sections of formalin fixed paraffin embedded tumor sections from 32 patients with invasive breast cancer enrolled for treatment at MD Anderson Cancer Center. For FISH, pretreatment of the slides with the paraffin pretreatment kit III (Abbott Laboratories) was performed followed by incubation using the ESR1/Cen6 probe set (Zytovision, Germany). The number of fluorescent signals for each the ESR1 and centromere 6 were counted in a minimum of 200 non-overlapping, intact nuclei. Nuclei were characterized as normal (two signals for each locus), monosomy (one signal for ER and two signals for the centromere) or trisomy (three signals for ER and two signals for the centromere). IHC on primary tumor tissue sections was carried out in a Bond-maX machine (Leica Microsystems) with primary ER antibody (clone 6F11, Novocastra) at dilution of 1:35 and antigen retrieval using citrate buffer. Nuclear positivity in the tumor cells was expressed as percentage and categorized as negative, low positive or positive based on nuclear staining of 0%, 1-10% and >10% respectively.

RESULTS: Of the 32 samples that were successful for both FISH and IHC, a comparison was made to determine concordance of FISH signals to ER IHC results. We calculated the percentage of cells having ≥3 ESR1 signals and those that contained <2 ESR1 signals in all 32 cases. Based on a FISH percentage cutoff of 2.0, cases could be classified into three groups: negative (n=7), equivocal (n=8) and amplified (n=10) with seven cases being discordant when compared to IHC results. The p-value for the ratio of ER negative to ER positive cohort was found to be statistically significant (p=0.026). Based on these criteria we observe a concordance of 75% between the two technologies.

CONCLUSION: 1) There is significant heterogeneity between the gene amplification status and protein overexpression of ESR1. 2) The gene status of ESR1 ranges from negative, equivocal and amplified in both ER negative and ER immunopositive cases. 3) The significance of heterogeneity at the ESR1 gene locus in ascertaining the prognosis and predictive response to antiestrogen therapy needs further evaluation in larger prospective clinical trials.

Hypothesis

Here we hypothesize that a positive correlation exists between ESR1 copy number and immunohistochemistry (IHC).

BACKGROUND

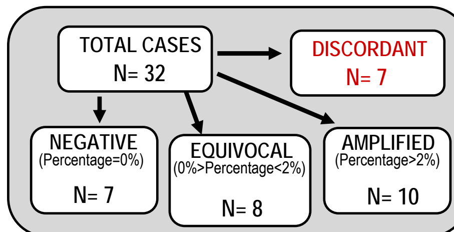
Estrogen receptor alpha (ER) is a widely accepted biomarker and great detail has been studied regarding the protein and RNA levels. It is estimated that up to 75% of breast cancers rely on estrogen receptor signaling for their means of growth and targeting this pathway has clear clinical efficacy[1]. Several treatments exist for estrogen receptor positive breast cancer patients that can alter estrogen receptor signaling. Selective estrogen receptor modulators (SERMs) such as tamoxifen act as a receptor antagonist, aromatase inhibitors, downregulation of estrogen receptor itself by antiestrogens such as fulvestrant, and even more drastic measures such as ovarian ablation[1]. More recently a number of studies have demonstrated amplification at the ESR1 locus, although the frequency of the amplification continues to be extensively debated[2-4].

ESR1

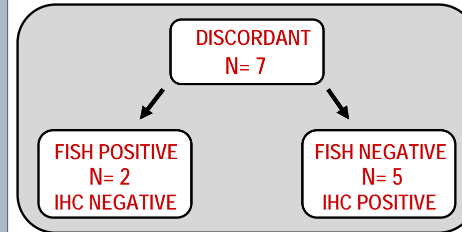
Tumor sections were analyzed for ESR1 copy number changes by FISH using an ESR1/CEN6 probe set from Zytovision. The ESR1 gene is located at 6q25 and is indicated by a green fluorochrome direct labeled probe and the centromere 6 probe is an orange fluorochrome direct labeled probe specific for the alpha satellite centromeric region of chromosome 6 (D6Z1).

CLASSIFICATION BASED ON FISH

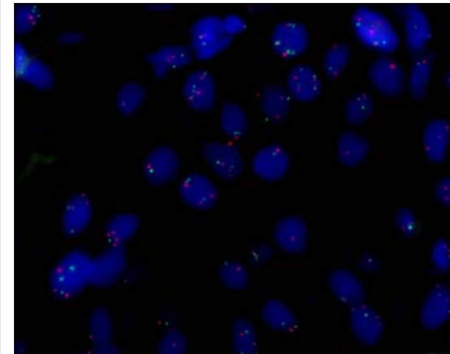
We calculated the percentage of cells having ≥3 ESR1 signals and those that contained <2 ESR1 signals with CEP6=2 in all 32 cases. Based on a FISH (≥3 ESR1 signals) percentage cutoff of 2.0, cases could be classified into three groups: negative (n=7; Percentage=0%), equivocal (n=8; 0%>Percentage<2%), and amplified (n=10; Percentage>2%). Seven cases were discordant when compared to IHC results. The p-value for the ratio of ER negative to ER positive cohort was found to be statistically significant (p=0.026). Based on these criteria we observe a concordance of 75% between the two technologies.



DISCORDANT RESULTS



HETEROGENEITY OF ESR1



Most changes in ESR1 signal patterns were also accompanied by the same changes in CEN6 signal patterns and in this small cohort of patients we did not observe a change in the ratio ESR1/CEN6 reported by other groups[2-4] (ratio range 0.92-1.11) to be classified by traditional amplification strategies.

CONCLUSIONS

1. There is significant heterogeneity between the gene amplification status and protein overexpression of ESR1.
2. The gene status of ESR1 ranges from negative, equivocal and amplified in both ER negative and ER immunopositive cases.
3. The significance of heterogeneity at the ESR1 gene locus in ascertaining the prognosis and predictive response to antiestrogen therapy needs further evaluation in larger prospective clinical trials.

METHODS & MATERIALS

Laboratory Information and Patients FISH and IHC for ESR1 was performed on adjacent sections of formalin fixed paraffin embedded tumor sections from 32 patients with clinical stage I-III breast cancer who had consented to participate in an IRB-approved study evaluating primary tumors markers at MD Anderson Cancer Center Adjacent sections from formalin fixed paraffin embedded blocks were cut at 5μm thickness and used for immunohistochemistry (IHC) and FISH.

Immunohistochemistry IHC on primary tumor tissue sections was carried out in a Bond-max machine (Leica Microsystems) following antigen retrieval using standard citrate buffer. Primary ER antibody (clone 6F11, Novocastra) was used at dilution of 1:35. Nuclear positivity in the tumor cells was expressed as percentage and categorized as negative, low positive or positive based on nuclear staining of 0%, 1-10% and >10% of the cells respectively.

Fluorescent in-situ hybridization Pretreatment of the slides with the paraffin pretreatment kit III (Abbott Laboratories) was performed followed by incubation using the ESR1/Cen6 probe set (Zytovision, Germany). The number of fluorescent signals for each the ESR1 and centromere 6 were counted in a minimum of 200 non-overlapping, intact nuclei. Nuclei were characterized as normal (two signals for each locus), monosomy (one signal for ER and two signals for the centromere) or trisomy (three signals or greater for ER and two signals for the centromere).

REFERENCES

1. Cleator SJ, Ahamed E, Coombes RC, Palmieri C. A 2009 update on the treatment of patients with hormone receptor-positive breast cancer. Clin Breast Cancer 2009; 9 Suppl 1: S6-S17.
2. Holst F, Stahl PR, Ruiz C, Hellwinkel O, Jehan Z, Wendland M, Lebeau A, Terracciano L, Al-Kuraya K, Jänicke F, Sauter G, Simon R. Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. Nat Genet. 2007 May;39(5):655-60.
3. Tomita S, Zhang Z, Nakano M, Ibusuki M, Kawazoe T, Yamamoto Y, Iwase H. Estrogen receptor alpha gene ESR1 amplification may predict endocrine therapy responsiveness in breast cancer patients. Cancer Sci. 2009 Jun;100(6):1012-7.
4. Nielsen KV, Ejlertsen B, Müller S, Møller S, Rasmussen BB, Balslev E, Lænkholm AV, Christiansen P, Mouridsen HT. Amplification of ESR1 may predict resistance to adjuvant tamoxifen in postmenopausal patients with hormone receptor positive breast cancer. Breast Cancer Res Treat. 2010 Jun 17.

CONTACT INFORMATION

Julie Ann Mayer, PhD
5810 Nancy Ridge Drive, Suite 150
San Diego, California 92121

T: 858.320.8200
F: 858.320.8225
E: jmayer@biocept.com