Comparison of fluorescence in situ hybridization of estrogen receptor (ESR1) with protein expression in invasive breast carcinoma

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ABSTRACT

INTRODUCTION: Evaluation of estrogen receptor (ESR1) status is recommended in 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 ESR1 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 pre-treatment kit III (Abbott Laboratories) was performed followed by incubation using the ESR1/Cent6 probe set (Zytovision, Germany). The number of fluorescent signals for each the ESR1 and centromere 6 were counted in a minimum of 500 non-overlapping, intact nuclei. Tumor sections were analyzed for ESR1 copy number changes by FISH using an ESR1/Cent6 probe set (Zytovision, Germany). ESR1 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 included samples with ≥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. The gene status of ESR1 ranges from negative, equivocal and amplified in both ER negative and ER immunopositive cases. 2) 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.

REFERENCES

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