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Expression of Estrogen Receptor Variants

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Abstract Recent data suggest that the expression of estrogen receptor (ER) variants may be common in clinical breast cancer. The significance of their expression is complicated by the fact that they are often coexpressed with wild-type ER in the same tumor. We have focused upon one such ER variant which lacks exon 5 within the hormone binding domain of the receptor. This deletion introduces a stop codon, resulting in a truncated ER of 40 kDa which is unable to bind hormone. We have been exploring the hypothesis that this variant may contribute to clinical antiestrogen resistance. Coexpression of the exon 5 variant along with wild-type ER in MCF-7 human breast cancer cells confers resistance to the commonly used antiestrogen, tamoxifen. In addition, we have observed that some metastatic breast lesions overexpress exon 5 ER deletional variant transcripts. We conclude that differences in the relative amounts of several ER variants in the same tumor may interact to determine hormonal responsiveness and metastatic behavior.

Key words: Breast cancer, ER mutations, tamoxifen resistance

The growth of many breast cancers appears to be dependent on the proliferative effects of estrogen, which correlates with the presence of a functional estrogen receptor (ER). The presence of ERs predicts the likelihood of benefit from antiestrogen treatment with agents such as tamoxifen. The recent tamoxifen overview analysis by the Early Breast Cancer Trialists' Collaborative Group [1] clearly demonstrates that tamoxifen is effective in delaying recurrence in the majority of women with breast cancer. However, most initially responsive tumors will eventually become resistant to tamoxifen treatment. Several hypotheses are being explored to explain tamoxifen resistance in human breast cancer; these include alterations in antiestrogen metabolism [2], alterations in oncogene expression, elevated growth factor and/or growth factor receptor expression [3], and alterations in the ER itself. This review will focus upon the latter hypothesis that specific alterations in the ER may be associated with hormone resistance in breast cancer cells.

Furthermore, it has been appreciated for some time that the presence of ERs predicts for improved disease-free survival. It is generally accepted that this clinical association is because the ER is a biomarker or manifestation of differentia-

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tion in breast tissues. We will present a model suggesting that specific ER variants may play a role not only in antiestrogen resistance and disease progression, but also in the earliest stages of breast carcinogenesis.

ESTROGEN RECEPTOR STRUCTURE/ FUNCTION AND TAMOXIFEN ACTION

Upon estrogen binding, the ER binds to DNA at specific sites, thereby influencing the expression of estrogen-responsive genes such as the progesterone receptor (PR), with resultant growth stimulation. ER appears to stimulate transcription through two distinct transactivation domains located in the amino terminal region (the hormone-independent domain) and the carboxy terminal region of the receptor [4]. Antiestrogens, such as tamoxifen, act as competitive inhibitors of estrogen binding. It has been suggested that tamoxifen resistance may be associated with a dysfunction of one or both of these ER transactivation domains. The carboxy terminus of the ER is the most complex of the receptor domains, being responsible for receptor dimerization, hormone-dependent transactivation, and estrogen binding. Residues within this region important for estrogen binding have recently been elucidated, suggesting that the estrogen binding pocket is composed of residues distributed throughout the primary sequence [5,6].

ISOLATION OF A TRUNCATED ER WITHIN THE HORMONE BINDING DOMAIN FROM HUMAN BREAST TUMORS

Due to the complexity and importance of the hormone-dependent transactivation domain of the ER, we focused first upon the carboxy terminal region, utilizing polymerase chain reaction (PCR) amplification of cDNA from breast tumors to individually clone and sequence ERs from breast tumors. We reasoned that there might be an ER alteration present which would result in a loss of hormone binding, such that the ER would become an independent stimulator of breast proliferation, perhaps through the action of the amino terminal hormone-independent transactivation domain of the receptor. To increase the likelihood of isolating such an ER variant, we began our search using specimens from patients who were ER-negative as assayed by classical ligand binding assays, but were nevertheless PR-positive. These patients have a higher proliferative rate as compared to tumors from ER-positive, PR-positive tumors and we hypothesized that this higher rate could be due to the presence of a dominantly acting ER inappropriately stimulating breast tissue proliferation.

We prepared oligonucleotide primers to the boundaries of the entire carboxy terminal hormone binding domain covering exons 4–8 of the receptor. These primers were then used to PCR amplify the region, followed by cloning, and sequence analysis from a small series of ER–/PR+ tumors [7]. We detected an ER variant which contained wild-type sequence for exons 4 and 6, but was precisely missing exon 5. Due to the introduction of a stop codon after amino acid 370 of the corresponding wild-type ER, this variant ER encodes a truncated out-of-frame protein of approximately 40 kDa. In addition, this ER variant appears to result from alternative RNA splicing, since genomic PCR amplification and sequence analysis of the exon/intron borders failed to detect mutations in the exon 4/5 and exon 5/6 consensus splice sites [Fuqua, unpublished results].

We have now begun a series of studies to determine the functional consequences of exon 5 ER deletion variant overexpression in human breast cancer cells [Fuqua, unpublished observation]. Since this variant is lacking a portion of the hormone binding domain implicated as being the most important region for estrogen/antiestrogen binding [5,8] we next questioned whether the variant might be able to stimulate proliferation, even though it cannot bind estrogen. Using a variety of reporter systems, we now know that the exon 5 ER deletion variant stimulates estrogen-responsive gene constructs even in the absence of hormone [7]. Thus, truncation beyond exon 4 in the ER results in a transcriptionally active, constitutive receptor whose activity is the result of the hormone-independent activity of the amino terminal region (which remains intact in the exon 5 ER deletion variant). But how does elevated expression of the exon 5 ER deletion variant affect response to antiestrogens such as tamoxifen, which exert their effects through the region that has been altered in the exon 5 deletion variant?

OVEREXPRESSION OF THE EXON 5 ER DELETION VARIANT RESULTS IN TAMOXIFEN-RESISTANT GROWTH

We have recently transfected the exon 5 ER deletion variant into MCF-7 human breast cancer cells expressing wild-type receptor, to address the important clinical question of tamoxifen resistance. Since this variant is missing the far carboxy terminus of the hormone-binding domain, one might predict that cells expressing it would be unresponsive to agents such as tamoxifen. We now have preliminary results suggesting that overexpression of the exon 5 deletion variant in MCF-7 cells confers tamoxifen-resistant growth *in vitro* [Fuqua, unpublished observation]. Interestingly, the exon 5 ER transfectants exhibit increased growth in soft agar. This latter result has stimulated us to explore the role of the exon 5 ER deletion variant in disease progression.

DO ER VARIANTS DRIVE PROLIFERATION?

We are now evaluating whether the exon 5 variant is overexpressed in tumors from tamoxifen-resistant patients using a semiquantitative cDNA PCR assay. We have data suggesting that many ER-positive breast tumors coexpress the exon 5 ER deletion variant along with wild-type ER. When we examine metastatic breast lesions, some of which are from tamoxifen-resistant patients, we find that the exon 5 ER deletion variant is elevated in some of these tumors. Although these results are obviously very preliminary, it is tempting to speculate that expression of this variant may be associated with disease progression and metastatic behavior. Obviously this hypothesis must be tested in a large clinical study.

We are also examining whether ER variants are present in some of the earliest stages of breast carcinogenesis, such as proliferative dis-

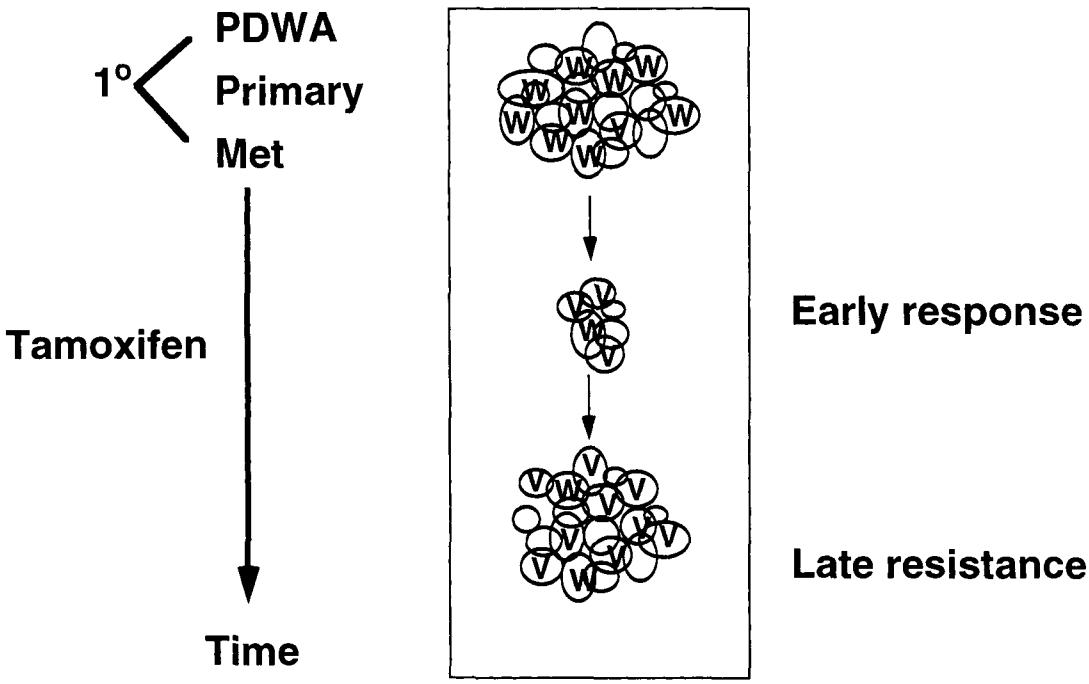

Fig. 1. A model for the involvement of ER variants in breast cancer progression and tamoxifen resistance. Cells overexpressing the exon 5 ER deletion variant are shown as cells labeled as "V". Cells within the tumor expressing wild-type ER are shown labeled with a "W". Lesions (1°) such as proliferative disease without atypia (PDWA), primary breast cancer, or metastatic breast cancer (met) are initially composed of cells containing both "W" and "V" ER-expressing cells. With time (disease progression) or under tamoxifen selection, the variant-expressing tumor cells become the predominant cell type.

ease without atypia (PDWA) or atypical ductal hyperplasia. Again, we are questioning whether ER variants, such as the exon 5 deletion variant, may be influencing inappropriate proliferation in these early breast lesions. To date we have examined four such lesions, and have found ER sequence changes in three. Although at present we do not know the functional significance of these sequence alterations, we are actively pursuing the hypothesis that ER variants may be one of the earliest genetic alterations in the series of genetic changes associated with breast carcinogenesis.

MODEL FOR THE INVOLVEMENT OF ER VARIANTS IN BREAST CANCER PROGRESSION AND TAMOXIFEN RESISTANCE

Our data would predict that patients with primary or metastatic breast cancer who initially display an early response to tamoxifen (shown in Fig. 1 as tumor cells with wild-type ER "W" in the tumor), and who initially contain low levels of the exon 5 variant, may eventually display late tamoxifen-resistant growth concomitant with the overexpression of the exon 5 ER deletion variant. This model predicts that there may be a dynamic process of cellular fluctuation, with some subclonal ER variant populations becoming predominant during progression and eventual tumor recurrence. Furthermore, this model predicts that ER variants may be present in some of the earliest breast lesions, such as PDWA. In this situation, ER may be the driving force behind early breast cell proliferation, setting the stage for other genetic changes to occur, and leading to eventual disease progression. This working model of course is extremely speculative, but presents a provocative hypothesis for the involvement of ER alterations in many steps of breast carcinogenesis. Thus, one of the most studied of the prognostic breast cancer biomarkers, the ER, may still hold many surprises in store for us, and many avenues for future clinical research.

CONCLUSIONS

We have focused on the hypothesis that alterations in the ER may be associated with hormone resistance and the clinical problem of antiestrogen resistance. We have identified an ER variant which is transcriptionally active in the absence of hormone, and which influences estrogen-independent cellular proliferation. Expression of this variant in breast cancer cells with wild-type ER expression results in resistance to tamoxifen. We are exploring its role, and the role of other ER sequence alterations in premalignant breast disease and breast cancer progression.

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