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TITLE: Prognostic Value of the K303R Estrogen Receptor α Mutation in Breast Cancer

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Prognostic Value of the K303R Estrogen Receptor α Mutation in Breast Cancer

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The estrogen receptor (ER) α plays a major role in breast tumor progression, we have recently discovered a somatic mutation (A908G) that leads to a lysine to arginine (K303R) amino acid change. Here we proposed to study if the K303R ERα mutation is prognostic clinical factor for invasive breast cancer. Our initial studies have indicated that the mutation is present in approximately 53% of invasive breast cancers. Additionally, the mutation was more prevalent in node-positive breast cancer versus node-negative breast cancer (85% vs. 38%, respectively). Specific Aim 1 has been completed and we are proceeding with specific Aim 2. Analyzing a larger patient population with long-term clinical follow-up. In the near future we will be begin creating the expression vectors and cell lines required to complete specific Aim 3. While our initial data suggest that the K303R ERα mutation may be involved in tumor progression and metastasis, completion of specific Aims 2 and 3 will confirm these results.

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estrogen receptor, mutation, prognostic factor, breast cancer

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INTRODUCTION
The estrogen receptor (ER) α plays a major role in breast tumor progression and is an important target for hormonal therapy. We have recently discovered a somatic mutation (A908G) in the hinge domain of ERα that leads to a lysine to arginine (K303R) amino acid change (1). Initially we have identified this mutation in 30% of breast hyperplasias and 60% of invasive breast cancers. The presence of the K303R ERα mutation confers hypersensitivity to estrogen thereby increasing transcriptional activity at very low concentrations of estrogen in in vitro assays. Additionally, we have recently shown that the presence of the K303R mutation creates a new PKA and PAK1 phosphorylation site (2). While this data would suggest that the K303R ERα expression would confer more aggressive phenotype for breast cancers, the prognostic value of this mutation remains to be determined. Additionally, the role, if any, of the K303R ERα mutation in breast tumor metastasis has not been defined. This proposal seeks to determine the clinical impact of the K303R ERα mutation on breast cancer prognosis through the following specific aims:

1. To determine the precise frequency of the mutation in breast cancer, and its association with node-positive cancers.
2. To ask how the mutation is correlated with proliferative potential and poorer prognosis of clinical breast cancer.
3. To study metastatic behavior of K303R-expressing breast cancer cell lines in in vivo models.

BODY
Specific Aim 1
To determine the precise frequency of the mutation in breast cancer, and its association with node-positive cancers, we have refined our microdissection and SNaPshot primer extension sequencing techniques. While 38% (10/26) of the node-negative breast cancers demonstrated the K303R ERα mutation, 85% of the node-positive breast cancers from node-positive breast cancers contained the mutation (p<0.001). While this mutation was present in 18 samples; ligand binding assays showed that only 16 of these samples were positive for ER expression. This result demonstrates that the mutation is present even in ERα negative tumors, and this may suggest that ERα expression may have been lost in some tumors during progression. Robertson (3) has estimated that about 20% of metastatic tumors from an ER-positive primary cancer can lose ER during metastatic progression.

Specific Aim 2
Determining if this mutation is correlated with poorer prognosis requires analysis of tumor samples with long-term clinical follow-up. As we have not begun to analyze these samples, we have not started work on this aim. We anticipate that completion of this Aim will confirm our initial studies.

Specific Aim 3
We have not started work on specific Aim 3.
KEY RESEARCH ACCOMPLISHMENTS
1. We have developed a highly sensitive and accurate method to detect the presence of the A908G mutation in ERα.
2. In our initial study, the presence of the mutation correlates with lymph node-positive breast cancer.

REPORTABLE OUTCOMES
1 meeting abstract (see bibliography)
1 review article (see bibliography)
Continued training of post doc

CONCLUSIONS
We have successfully completed Specific Aim 1 and are beginning to work on specific Aims 2 and 3. We have developed and refined our sequencing techniques and found preliminary results demonstrating a mutation frequency of approximately 53%. Additionally, this mutation is more prevalent in node-positive invasive breast cancers. We will now proceed with specific Aim 2, analyzing a larger patient population, with long-term clinical follow-up. As our early data indicates that the mutation is present in more advanced lymph node-positive breast cancers, it will be exciting to analyze this mutation with known clinical parameters such as tumor size, and proliferation (Ki67 or S phase fraction), as well as recurrence free survival and overall survival as outlined in specific Aim 2. This data suggests that this mutation may play an important role in breast cancer progression and metastasis. The funds for this fellowship have been well spent in determining the important clinical role of this common ERα mutation.

BIBLIOGRAPHY


REFERENCES


APPENDICES

Figure Legends
Figure 1. Representative sequencing chromatograms from three invasive ductal breast tumors. Tumor 1 wild-type homozygote (panels A-C), tumor 2 heterozygote (panels D-F), and tumor 3 mutant homozygote (panels G-I) were sequenced using manual radioactive (panels A, D, G), *SNaPshot* (panel B, E, H), and dye terminator (panel C, F, I) using forward sequencing primers. Arrows indicate the WT or mutant peaks. The three-base pair combination GAA is overlined, and the 908 base position is underlined.