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13. SUPPLEMENTARY NOTES					
14. ABSTRACT 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 ER α mutation is prognostic clinical factor for invasive breast cancer. We have determined that the mutation is present in approximately 56% of invasive breast cancers and its' expression correlates with lymph node involvement. Expression constructs containing the wild-type or mutant ER α fused to a yellow-fluorescent protein have been developed and stably transfected into MCF-7 breast cancer cells. In vivo nude mouse studies are underway to analyze experimental metastases. Specific Aim 1 has previously been completed and the mutation frequency has been determined in Aim 2. Additional analysis of clinical parameters is ongoing. Specific Aim 3 is underway and mice will be analyzed for experimental metastases in the near future.					
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Annual Report

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 ER α 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 ER α 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 ER α 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 ER α mutation in breast tumor metastasis has not been defined. This proposal seeks to determine the clinical impact of the K303R ER α 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

Last year we reported that the K303R ER α mutation was present in approximately 53% of invasive breast cancers. While the mutation was present in 38% of node-negative cancers, 85% of node-positive invasive breast cancers demonstrated the presence of the mutation. As we reported that we had completed specific Aim 1, we have not worked on this specific Aim in the current reporting period.

Specific Aim 2

To determine if this mutation is correlated with poorer prognosis, we have begun to analyze patient samples with the known clinical variables and follow-up. Utilizing *SNaPshot* primer extension analysis we have found the mutant ER α in 163 out of 293 (56%) primary invasive breast cancers. Thus, we have determined that this ER α mutation has a relatively high frequency in invasive breast cancers. Analysis of these samples with the known clinical variables and follow-up is underway, and thus, not reportable at this time. However, this larger study has confirmed our initial studies in specific Aim 1. The presence of the A908G ER α mutation statistically correlated with lymph node involvement and the results are listed in Table I. Further analysis of clinical variables and follow-up is ongoing.

Specific Aim 3

To study metastatic behavior of K303R-expressing breast cancer cell lines in *in vivo* models, we have developed expression vectors with either the K303R ER α or the wild-type ER α fused to a yellow fluorescent protein. These have been named YFP-WT ER α and YFP-K303R ER α , respectively. MCF-7 cells were stably transfected and individual clones were isolated and expanded with G418 selection. Stably transfected clones were screened for expression of the exogenous ER α by immunoblot analysis (Figure 1). One clone stably expressing YFP-WT ER α (clone WT-ER α) and one clone stably expressing YFP-K303R ER α (clone KR-ER α) with similar levels of the exogenous YFP-ER α and endogenous ER α were chosen for further analysis. MCF-7 breast cancer cells expressing the K303R ER α mutation are currently being assayed for their ability to form experimental metastases in immunocompromised mice. WT-ER α and KR-ER α cells (1×10^6 cells per animal) were injected into the tail vein of nude mice. The mice will be sacrificed 6 months post injection and number of lung metastases will be quantitated. This experiment is currently ongoing.

KEY RESEARCH ACCOMPLISHMENTS

1. We have developed a highly sensitive and accurate method to detect the presence of the A908G mutation in ER α .
2. The A908G mutation is present in 163/293 (56%) of invasive breast cancers examined.
3. The presence of the mutation correlates with lymph node involvement.
4. We have developed expression vectors with either the K303R ER α or the wild-type ER α fused to a yellow fluorescent protein.
5. MCF-7 cells are stably expressing either the YFP-WT ER α or YFP-K303R ER α .
6. Immunocompromised mice have been injected with WT-ER α or KR-ER α cells to analyze experimental metastases.

REPORTABLE OUTCOMES

1 meeting abstract (see bibliography)
1 book chapter (see bibliography)
1 manuscript in preparation
Continued training of post doc

CONCLUSIONS

Last year we reported that we have successfully completed Specific Aim 1. We have sequenced the additional samples stated in specific Aim 2 and are currently analyzing the clinical variables and follow-up. Initially we have determined that the mutation is present in 56% of breast cancer samples and correlates with lymph node involvement. Continuing analyses of additional clinical parameters is ongoing. To study the metastatic behavior of K303R ER α expressing breast cancer cells, we have developed expression vectors and stably transfected these into MCF-7 breast cancer cells. While we have injected cells into the tail vein of nude mice, enough time has not elapsed to analyze these mice for lung metastases. While a high number of invasive breast cancers demonstrate the presence of this mutation and it's correlation with lymph node status is very exciting, our continued analysis will determine if the K303R ER α is a new prognostic factor for breast cancer. This mutation will undoubtedly become an important factor in treatment

decisions. While the studies on Specific Aims 2 and 3 are still ongoing, our preliminary data is very intriguing. The funds for this fellowship are being well spent in determining the important clinical role of this common ER α mutation.

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APPENDICES

Table I Correlation Between the A908G ER α mutation and lymph node status

Variable	Mutation status (%)	p-value
Nodal Group		<0.0001
0	42	
1-3	72	
>3	80	

Figure Legends

Figure 1. MCF-7 cells stably overexpressing wild-type ER α (clone WT-ER α) or the mutant K303R ER α (clone KR-ER α) were serum starved, then treated with or without 10^{-9} M estrogen for 10 minutes prior to lysis. Shown is immunoprecipitated ER α (lanes 1-4) and whole cell lysates (lanes 5-8) that have been immunoblotted for ER α .

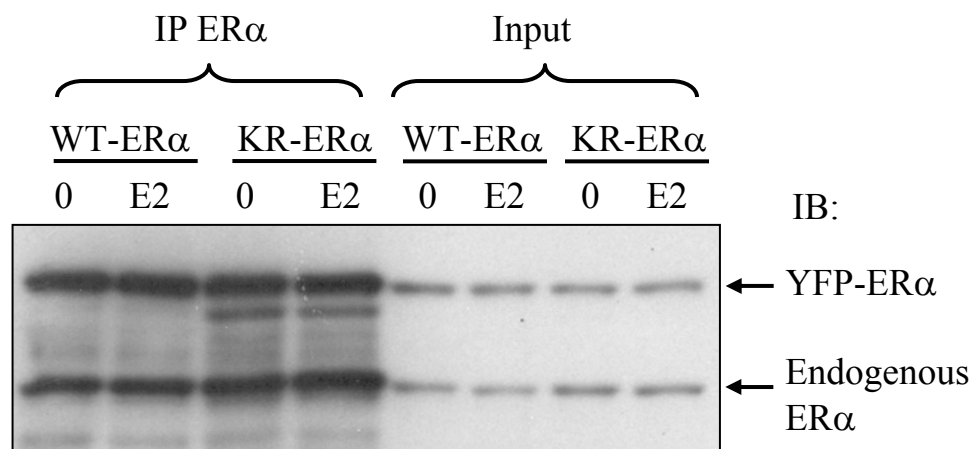


Figure 1