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13. ABSTRACT (Maximum 200 Words) The induction of human breast cancer involves the complex interplay of hormones and growth factor Receptors. The major focus of our DOD sponsored research program is to investigate the interaction of the ER α and erbB-2 receptor in the induction of breast cancer. In addition, we are also interested in the involvement of ErbB-2 coupled signaling pathways such as c-Src and Akt-1 in ER α induced mammary tumors. To accomplish this we have recently derived transgenic mice that expressed high levels of ER α in the mammary epithelium. To explore the significance of erbB-2c-Src and Akt-1 in mammary tumor progression we have recently initiated intercrosses between mice expressing these signaling molecules in the mammary epithelium and the MMTV/ER α strains. The results of these analyses should provide important insight in the mechanism by which erbB-2 and ER α communicate in the induction of breast cancer.				
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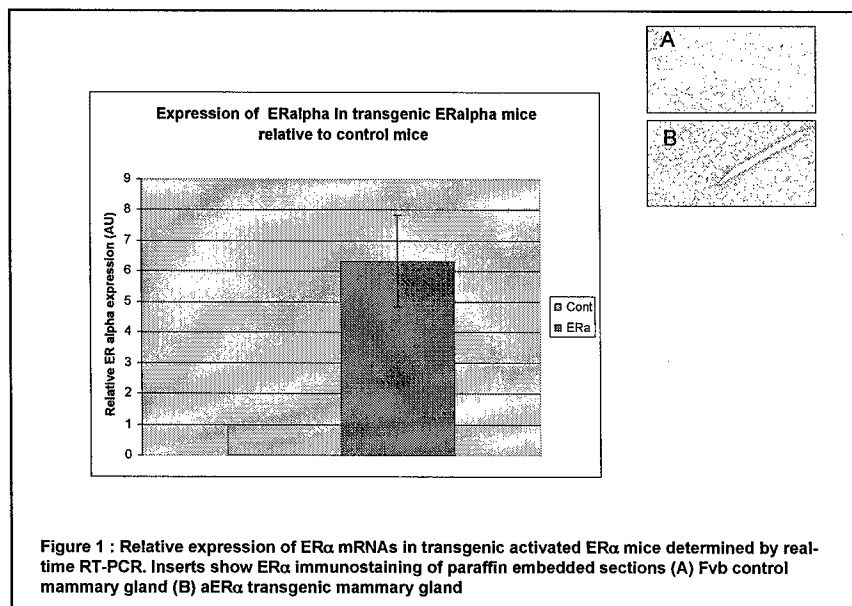
Introduction

The major goal of our DOD sponsored research program has been directed towards understanding the importance of crosstalk between the erbB-2 receptor tyrosine kinase and ER α receptors. The basis for these experiments derives from the observation that in addition to well documented role of ER α as a transcriptional activator, ER α has been shown to mediate a number of non-genomic effects that involve physical complexes with the c-Src tyrosine kinase (2). Given that c-erbB-2 is also known to activate c-Src tyrosine kinase (3, 4), it conceivable that c-Src may play a critical role in mediating cross-talk between ER α and ErbB-2. To further test the importance of ER α in ErbB-2 mediated mammary tumorigenesis, one of the major goals of this proposal was investigate whether mammary epithelial specific expression of ER α may modulate mammary tumor progression in the MMTV/ErbB-2 strains. To accomplish these goals, we have recently derived transgenic mice that express an constitutively activated version of ER α under the transcriptional control of the MMTV promoter. To further explore the significance of cross talk of ER α and ErbB-2 in the last renewal period we have crossed the MMTV/Era mice with separate strains of transgenic mice expressing ErbB-2 under the its endogenous or MMTV promoters. In addition, we also assessed whether ErbB-2 coupled signaling molecules such as c-Src and Akt could act synergistically with ER α to accelerate mammary tumor progression. This was accomplished by interbreeding the MMTV/activated ER α mice with separate strains of transgenic mice expressing activated forms of Akt-1 or c-Src under the transcriptional control of the MMTV promoter.

Body

Mammary specific expression of ER α is not associated with mammary tumor induction.

As reported in our first annual report, thee transgenic mice express high levels of ER α in the mammary epithelium. To confirm that these strains expressed high levels of ER α , we performed real time polymerase chain reaction (RT-PCR) on RNA derived from either MMTV/ER α or control FVB mice. The results revealed that MMTV/ER α mice expressed elevated levels of ER α transcript in the mammary epithelium. Consistent with RT-PCR results, immunostain analyses with ER α specific antisera revealed robust nuclear staining (Figure1). Despite the high levels of ER α expression in the mammary



epithelium, female mice carrying the MMTV activated ER α have yet to develop mammary tumors.

Generation of bitransgenic animal co-expressing Neu, Akt-1 and c-src with activated ER α in the mammary epithelium

Genotype	Number of transgenic females	Range of ages
aER α * ¹	85	2 to 12 months
Strain 02-351-5	45	2 to 12 months
Strain 02-346-1	20	2 to 12 months
Strain 02-347-2	20	2 to 12 months
aER α /NeuNDL2-5* ²	20	2 to 8 months
aER α /FloxNeoNeuNT/MMTV-Cre* ³	3	2 to 4 months
aER α /Src2* ⁴	24	2 to 10 months
aER α /Akt7* ⁵	5	2 to 6 months

*1 Activated form of human Estrogen Receptor alpha under the control of the MMTV promoter
 *2 Activated ErbB2 under the under the control of the MMTV promoter
 *3 Activated ErbB2 under the control of its endogenous promoter
 *4 Activated form of Src under the control of the MMTV promoter
 *5 Activated form of Akt-1 under the control of the MMTV promoter

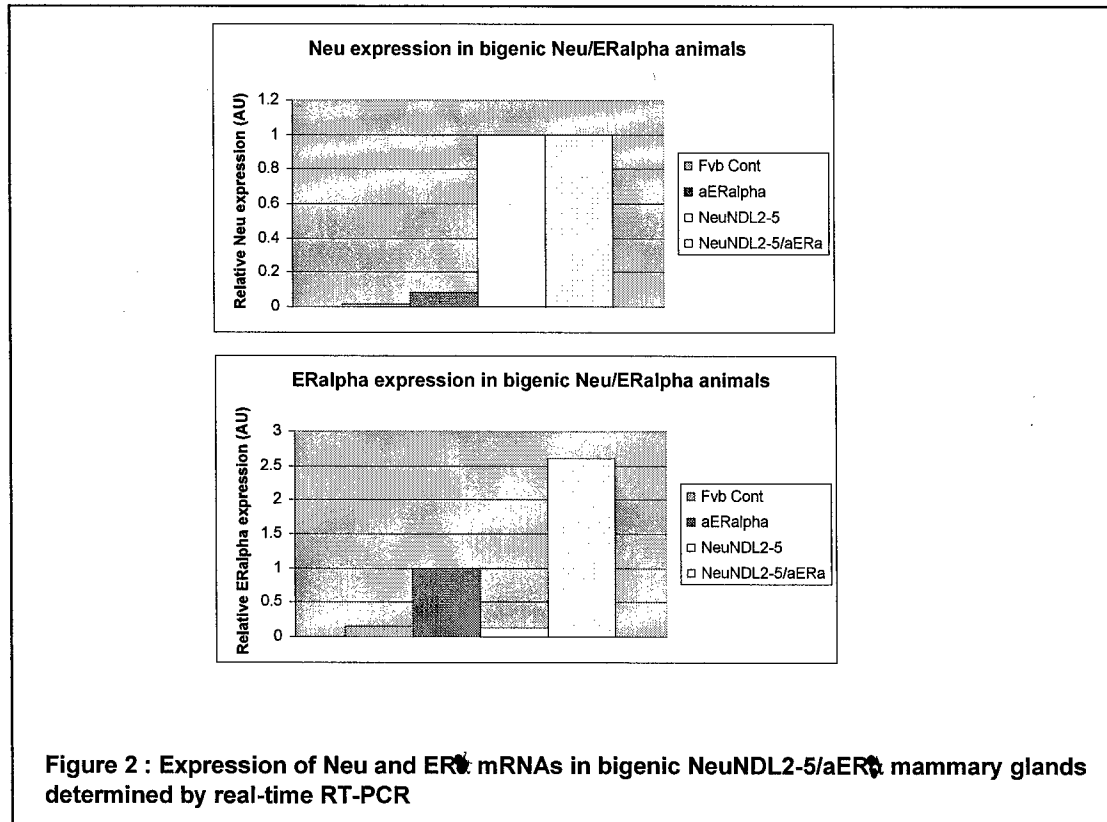
Table 1 : Summary of generated transgenic mice

Genotype	Tumor onset (days)	Total number of animals
NeuNDL2-5	168	3
NeuNDL2-5 / aER α	170	5

Table 2 : Tumor onset in bigenic animals compared to activated Neu transgenic mice (NeuNDL2-5)

To assess whether co-expression of activated ER α and Neu could impact on tumor induction, the MMTV/ER α were interbred either with separate strains of mice carrying MMTV/activated erbB-2 (Neu NDL2-5, Table1) or mice carrying activated erbB-2 under the endogenous erbB-2 promoter (Flox Neu-NT/MMTV/cre, Table #1). To date we have generated a cohort of 20 virgin females harboring both MMTV/activated erbB-2 and MMTV/ER α mice and 3 females co-expressing ER α in the context of endogenous ErbB-2 promoter (Table #1). In addition to these crosses we have also generated transgenic crosses in which key downstream signaling pathways such as c-Src and Akt-1 are coexpressed in the mammary epithelium with ER α (ER α /c-Src, ER α /Akt7, Table#1). Although we are still in the early stages of analyses for these various crosses, there are

preliminary indication that co-expression of erbB-2 and ER α does not significantly impact on tumor induction in these strains since the bi-genic mice are developing mammary tumors within a similar latency period (Table #2).



The lack of accelerated tumor phenotype was not due to lack of expression of either ER α or Neu as the bigenic mammary glands expressed elevated levels of Neu and ER α transcript compared to the FVB control tissues (Figure 2). Histological analyses of mammary tumors derived from the Neu/ER α revealed that they had comparable pathological phenotypes.

Key Research Accomplishments

1. Generation of bigenic female mice coexpressing ER α and ErbB-2 in the mammary epithelium.
2. Initial characterization of bigenic ER α and erbB-2 tumor phenotype.
3. Generation of bi-genic strains co-expressing activated c-Src and ER α in mammary epithelium
4. Generation of bi-genic strains co-expressing Activated Akt-1 and ER α

Reportable Outcomes

Derivation and characterization of the MMTV/ER α strains.

Conclusions

Although these analyses are based on relatively small cohorts of transgenic animals (Table #2), the future characterization of remaining cohort of animals should provide important insight into the role of ER α in ErbB-2 induced mammary tumor progression. Given the that ER α is known to be involved in the transcriptional regulation of erbB-2 promoter (1), the most informative cross will be female mice expressing ER α in the context of endogenous erbB-2 promoter (Table #1). While we have a cohort of only 3 female, we are in the process of expanding these mice.

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