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PRINCIPAL INVESTIGATOR: Yi Li, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, TX 77030

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Yi Li, Ph.D.

Email: liyi@breastcenter.tmc.edu

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14. ABSTRACT
The goal of this study is to test whether ER– breast cancers arise from ER– or ER+ mammary cells. We specifically hypothesize that ER is absent in the originating cell of ER-negative breast cancer. Although until now it has been technically difficult to test it, we have developed a unique mouse model based on the RCAS-TVA technology that allows us to trace the ER status of the cancer-originating cell. It is now possible to test this hypothesis in experimental mice. In this initial funding period, we have generated mouse lines and viruses for the experiments proposed, and established experimental conditions. In addition, we have found in the course of th

15. SUBJECT TERMS
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BC060332: ER/PR status of the originating cell of ER-negative breast cancer

Yi Li, Ph.D.

INTRODUCTION

The goal of this study is to test whether ER– breast cancers arise from ER– or ER+ mammary cells. We specifically hypothesize that ER is absent in the originating cell of ER-negative breast cancer. Although until now it has been technically difficult to test it, we have developed a unique mouse model based on the RCAS-TVA technology that allows us to trace the ER status of the cancer-originating cell. It is now possible to test this hypothesis in experimental mice.

BODY

Task 1. Determine whether ER– mammary tumors arise from a PR– normal mammary precursor cell.

Strategy. We will infect mammary glands in the MMTV-tva/$PR^{Cre/+}$ female mice with RCAS viruses that express both an oncogene and a GFP reporter that is flanked by the loxP recombination sites and thus can be deleted by the Cre expressed from the PR promoter. Expression of PR in any infected cell will delete proviral GFP and cause this cell and all of its progeny to stop expressing GFP. Thus, if the cell destined to become PR– mammary cancer does not express PR any time prior to full transformation, tumor cells in the resulting cancer will retain GFP. Consequently, GFP expression in these PR– tumors will demonstrate that the tumors arise from a PR– cell. Conversely, lack of GFP in the resulting tumors will reveal that PR is expressed some time in the transition from a normal epithelial cell to malignancy.

We have generated strong evidence that in MMMTV-tva mice, RCAS can infect both ER+ and ER- cells. Using co-immunofluorescent staining 2.5 days following intraductal injection of RCAS-β-actin-HA into adult tva transgenic mice, 15%(±17%) β-acitn-HA+ cells were found to express ER, and 85% were negative for ER. Similar results were obtained using RCAS-GFP to infect these mice (Figure 1).

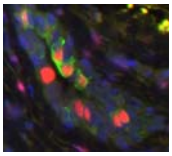

Figure 1. GFP is detected in ER+ and ER- mammary cells 4 days following infection of MMTV-tva adult mice with RCAS-GFP virus. GFP (green), ER (red).

We have imported $PR^{Cre/+}$ mice [1] and bred with our MMTV-tva mice.

We have constructed and tested RCAS expressing PyMT and floxed IRES-GFP, and found that this GFP expression is lost upon Cre expression in cultured cells. This virus can infect MMTV-tva mice, and the resulting tumors express both PyMT and GFP. However, some of the tumor cells lost GFP although Cre is not absent. We are testing whether this virus is not stable, and is considering the use of a different viral vector. Our RCAS env pseudotyped HIV vector [2] is an alternative.

We have constructed and tested RCAS virus expressing Neu and floxed IRES-GFP. The virus can infect in vivo, but cannot induce tumors within 6 months. We are currently reconstructing this virus to improve its potency. We will also consider cloning this insert in the pseudotyped HIV vector.

We have constructed RCAS viruses expressing a stable mutant of c-Myc (T50A)[3]. We will next append IRES-floxed GFP c-Myc. If this insert is not stable, we will move it into the pseudotyped HIV vector.

Task 2. Determine whether ER– mammary tumors arise from an ER– precursor cell in the normal mammary epithelium.

We proposed to create ER-Cre BAC transgenic mice, breed with MMTV-tva mice, and use the strategy in Task 1 to test whether ER– mammary tumors arise from an ER– precursor cell in the normal mammary epithelium.

The Cre protein is difficult to detect in vivo. Therefore, we first cloned a reporter gene (tva encoding a protein product that can be easily detected by IHC) into the best ER BAC we could find, and made two BAC transgenic founders. However neither founder line expressed tva at detectable levels. Therefore, the best ER BAC clone available at this time cannot express an exogenous gene at a detectable level. Thus, the knock-in approach appears to be a necessary alternative. We are in the process of starting this approach with the help of our knockout mouse facility.

Task 3. Discover molecular differences in response to oncogene activation between ER– and ER+ mammary cells in the normal mammary gland.

We have established proper cell isolation method and FACS protocol for primary mammary cells infected by RCAS or RCAS-env pseudotyped HIV virus.

Other findings from work not initially proposed:

Somatic activation of growth factor signaling induces estrogen-independent tumor initiation. Inhibition of estrogen signaling is effective in the prevention of some ER^+ breast cancers. However, there is no apparent preventive effect on the incidence of ER^- tumors. It remains unknown why attenuation of estrogen signaling prevents certain breast cancers but not others. Knowledge of the mechanisms underlying estrogen-independent tumor initiation could lead to better preventive therapies. Estrogen's mitogenic effects in the normal mammary gland are thought to be mediated via a paracrine mechanism that ultimately leads to proliferation of ER^- cells via activation of growth factor signaling pathways. Therefore, oncogenic activation of growth factor signaling pathways in normal mammary epithelial cells could render tumor initiation estrogen-independent. Our laboratory has developed a method of introducing oncogenes into a small number of individual cells within a developmentally normal mouse mammary gland with temporal control, thus allowing us to closely recapitulate the initiation of cancer as it occurs in humans. Using this model, we have induced both ER^+ and ER^- mammary tumors through activation of growth factor signaling pathways by overexpressing the PyMT or Her2/Neu oncogenes. Estrogen deprivation was unable to prevent or delay tumors induced by both oncogenes, suggesting that somatic activation of growth factor signaling pathways induces estrogen-independent tumor initiation. Furthermore, we found that Her2/Neu causes normally quiescent ER^+ cells to proliferate. Most of these cells, as well as Her2/Neu-overexpressing ER^- cells, no longer depend on estrogen signaling for proliferation. Therefore, these estrogen-independent Her2/Neu-overexpressing cells may be the precursors to the resulting tumors.

KEY RESEARCH ACCOMPLISHMENTS:

- Produced strong evidence that both ER+ and ER- cells can be infected by RCAS viruses.
- Demonstrated that somatic activation of growth factor signaling induces estrogen-independent tumor initiation

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- somatic activation of growth factor signaling induces estrogen-independent tumor initiation

CONCLUSIONS: We have created valuable reagents, and found that somatic activation of growth factor signaling induces estrogen-independent tumor initiation.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine*, etc.).

1. Soyal SM, Mukherjee A, Lee KY, Li J, Li H, Demayo FJ, Lydon JP. Cre-mediated recombination in cell lineages that express the progesterone receptor. Genesis 2005; 41:58-66.
2. Siwko S, Bu W, Gutierrez C, Lewis BC, Jechlinger M, Schaffhausen B, Li Y. Lentivirus-mediated oncogene introduction into mammary cells in vivo induces tumors. neoplasia 2008; In press.
3. Yeh E, Cunningham M, Arnold H, Chasse D, Monteith T, Ivaldi G, Hahn WC, Stukenberg PT, Shenolikar S, Uchida T, Counter CM, Nevins JR, Means AR, Sears R. A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nat Cell Biol 2004; 6:308-18.

APPENDICES

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