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13. ABSTRACT (Maximum 200 Words) A major problem in breast cancer treatment and the leading cause of mortality is invasion and metastasis of primary breast tumors. The cell type from which the tumor arises may dictate its potential for aggressive behavior. The mammary gland consists of different cell types including the cap cell; a less differentiated, highly proliferative cell basally located in the terminal end bud (TEB) of the murine mammary gland. The TEBs invade the fatty stroma of the pubertal gland establishing the ductal network. These specialized structures are reported to be targets for carcinogen-induced DNA damage. Their human counterparts are called intralobular ducts and are also sites of cancerous lesions. We hypothesize that genetic change specific to the cap cell population of the TEB will lead to aggressive tumors and metastatic disease. P-cadherin is normally expressed in the cap cells of the TEB and its progenitors. To test whether the less differentiated Pcadherin-positive cells have greater metastatic potential, the neu/HER-2 proto-oncogene will be targeted specifically to the cap cell population using the endogenous P-cadherin promoter. The goal of this research is to determine whether the highly proliferative and invasive cap cell population is a target for metastatic breast cancer.			
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusions.....	6
References.....	6
Appendices.....	6

INTRODUCTION:

A major problem in breast cancer treatment and the leading cause of mortality is invasion and metastasis of primary breast tumors. The cell type from which the tumor arises may dictate its potential for aggressive behavior. The mammary gland consists of different cell types including the cap cell; a less differentiated, highly proliferative cell basally located in the terminal end bud (TEB) of the murine mammary gland. The TEBs invade the fatty stroma of the pubertal gland establishing the ductal network. These specialized structures are reported to be targets for carcinogen-induced DNA damage. Their human counterparts are called intralobular ducts and are also sites of cancerous lesions. We hypothesize that genetic change specific to the cap cell population of the TEB will lead to aggressive tumors and metastatic disease.

P-cadherin is normally expressed in the cap cells of the TEB and its progenitors. To test whether the less differentiated P-cadherin-positive cells have greater metastatic potential, the neu/HER-2 proto-oncogene will be targeted specifically to the cap cell population using the endogenous P-cadherin promoter. To avoid any toxic effects of the oncogene prior to mammary gland development, we will utilize an inducible expression system in which transgene expression can be tightly regulated *in vivo*. Tumor development will be examined in these animals and tumor pathology will be compared to human breast tumors as well as transgenic models. The goal of this research is to determine whether the highly proliferative and invasive cap cell population is a target for metastatic breast cancer.

BODY:

Our research accomplishments for this Exploration Award are outlined below.

Task 1. Generate an inducible expression system to activate genes in a unique subset of mammary cells.

- a. Using homologous recombination in murine embryonic stem (ES) cells, place the reverse tetracycline transcriptional activator, rtTA, under the control of the endogenous P-cadherin promoter.

The following targeting vector containing the rtTA cassette was transfected into ES cells by electroporation (Fig. 1A). To promote mRNA stability an intronic sequence (not shown in the schematic) is present between rtTA and polyadenylation signal (pA). We identified ES clones that had undergone homologous recombination at the P-cadherin locus by Southern blotting (Fig. 1B).

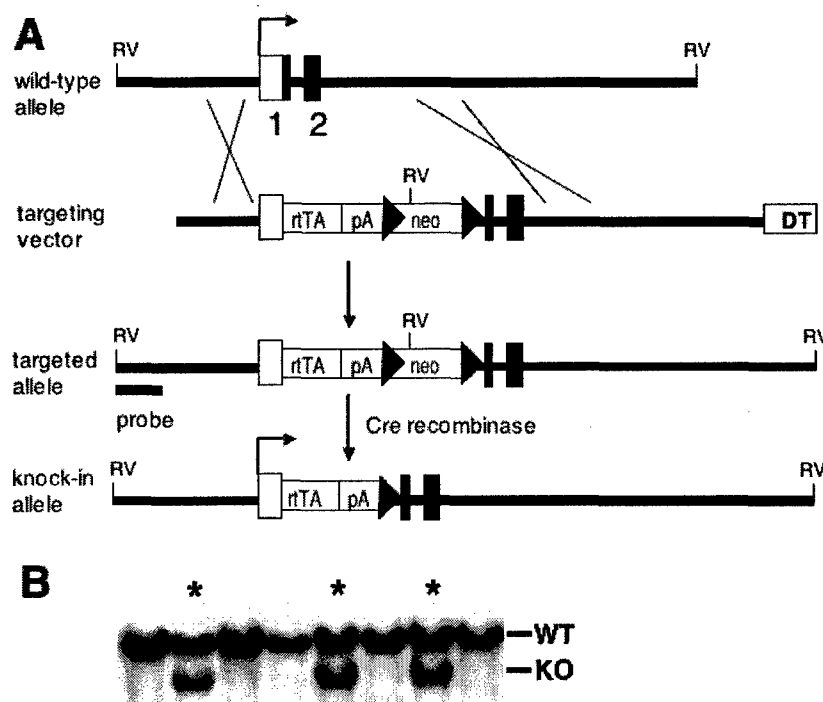


Figure 1. Generation of a P-cadherin/rtTA knock-in allele. A) Schematic representation of the expected gene replacement at the P-cadherin locus. Exons are represented as closed boxes. The PGK-neomycin resistance cassette and the PGK-diphtheria toxin cassette are designated as neo and DT, respectively. The ATG of the P-

cadherin gene was replaced with the initiator ATG in the rtTA gene. B) Southern blot analysis of wild-type and targeted (asterisk) ES cell clones. The flanking probe used for screening ES cell clones is shown (probe). After digestion with EcoRV, the wild-type and targeted allele result in an 18 and 11 kb DNA fragment, respectively. LoxP sites are represented by arrowheads. Restriction endonuclease sites: RV, EcoRV.

b. Generate mice containing the P-cadherin/rtTA knock-in allele.

Targeted P-cadherin/rtTA knock-in ES clones were injected into blastocysts by the Transgenic Core Facility at the University of Pennsylvania. Germline transmission of the mutant allele was achieved. The neomycin selection cassette was deleted from the locus by breeding animals to the protamine-Cre transgenic line. Expression of rtTA from the P-cadherin locus was examined by Northern blot analysis (Fig. 2). Expression was observed in placenta, a tissue that expresses endogenous P-cadherin at high levels. The mammary gland consists of many cell types of which the P-cadherin expressing cells make up a small fraction, therefore the Northern signal was very weak.

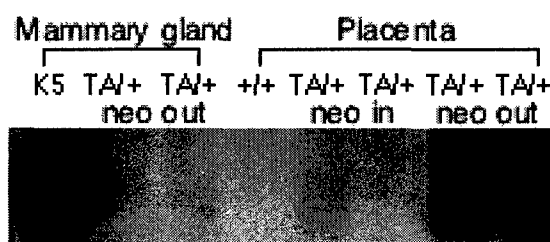


Figure 2. Northern blot analysis of P-cadherin/rtTA knock-in mice. RNA was isolated from mammary gland and placenta from rtTA (TA) mice with neomycin cassette (neo) present (in) or deleted (out) from the knock-in allele. The Northern blot was probed with rtTA gene. The rtTA gene was expressed at high levels in the placenta with lower levels found in the mammary gland. Note removing neo from the gene resulted in significantly greater expression of rtTA. Keratin-5/rtTA (K5) was a positive control. Wild-type (+/+) placenta served as a negative control.

To determine if rtTA protein was present, we examined placenta by Western blot (Fig. 3). We did not observe rtTA protein in the mice. This result suggested that rtTA mRNA was not translated into protein. In case, rtTA protein was present at levels below our detection, we decided to perform the experiment below.

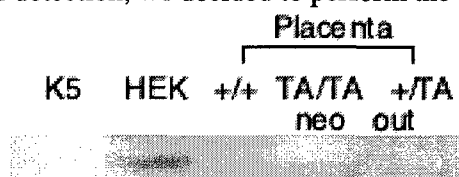


Figure 3. Western blot analysis of P-cadherin/rtTA knock-in mice. Protein lysates were isolated from placenta containing either one copy of the rtTA insertion (+/TA) or homozygous (i.e. 2 copies) of the knock-in allele (TA/TA). In neither case did we observe rtTA protein. Protein lysates from K5/rtTA mice and HEK cells transfected with rtTA expression cassette were used as positive controls. The blot was probed with VP16 antibody.

c. Confirm the cell type-specificity of the P-cadherin/rtTA transgene by breeding the mice to the tet operon/LacZ indicator strain.

In order to further characterize the P-cadherin/rtTA mice, the knock-in mice were bred to mice containing a reporter construct, pTetO-LacZ, in which expression of the LacZ gene is driven by a tetO-containing promoter cassette (provided by Dr. Lewis Chodosh, University of Pennsylvania). This construct permits the inducible expression of LacZ in response to tetracycline in cells expressing the rtTA transcriptional activator. Bitransgenic mice and nontransgenic littermates were given doxycycline in the drinking water. Mammary glands from wild-type and bitransgenic mice were harvested after treatment with doxycycline, as well as breast tissue from a bitransgenic littermate not treated with doxycycline. In situ histochemical staining for β -galactosidase activity in mammary glands from doxycycline treated and untreated bitransgenic animals were negative in both fixed and frozen sections

of tissue. Furthermore, we examined placenta from these animals, which expressed the rtTA mRNA at high level, and it was also negative.

Task 2. Examine mammary gland development and tumorigenesis in mice expressing the neu/HER-2 proto-oncogene in cap cells and their progenitors.

The animal model generated in Task 1 was necessary for Task 2, therefore this experiment was not performed.

KEY RESEARCH ACCOMPLISHMENTS:

1. Generated and characterized a novel P-cadherin/rtTA knock-in allele.

REPORTABLE OUTCOMES: We developed genetically modified mice. Unfortunately, the animals did not express functional rtTA protein as we had hoped.

CONCLUSIONS: Although rtTA mRNA was present in these animals, we did not observe protein or transactivation (i.e. β -galactosidase activity) in the bitransgenic animals. We do not understand why this genetic strategy did not work, however we can speculate on one possibility. The introduction of the rtTA cassette directly into exon 1 may affect the Kozak site leading to inefficient translation of the mRNA resulting in no protein. A future strategy for this knock-in approach might include moving the rtTA cassette away from exon 1, perhaps into exon 3, and include an Internal Ribosome Entry Site (IRES) to reinitiate translation. In this way, we would avoid the 5' untranslated region of the P-cadherin gene including the Kozak site.

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APPENDICES: None

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