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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

The objective of this research was to identify mouse mammary gland stem cells, with the ultimate goal being their isolation. We hypothesized that mammary gland stem cells can be identified by generating transgenic mice using a LEF/TCF-dependent reporter gene as a marker. Furthermore, by using green fluorescent protein (GFP) as the reporter, putative stem cells could be isolated by fluorescence activated cell sorting. The Specific Aims of the proposal were: 1) Generate transgenic mouse lines expressing GFP from a LEF/TCF-dependent promoter; 2) Determine sites of GFP transgene expression during mammary gland development, involution and remodeling. The ability to identify and isolate stem cells would simplify their characterization as well as the study of factors necessary for proliferation, differentiation, and susceptibility to transformation. Aim 1 was successfully completed, resulting in the generation of 4 Lef-GFP transgenic founders.

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Introduction

The objective of this research was to identify mouse mammary gland stem cells, with the ultimate goal being their isolation. We hypothesized that mammary gland stem cells can be identified by generating transgenic mice using a LEF/TCF-dependent reporter gene as a marker. Furthermore, by using green fluorescent protein (GFP) as the reporter, putative stem cells could be isolated by fluorescence activated cell sorting. The Specific Aims of the proposal were: 1) Generate transgenic mouse lines expressing GFP from a LEF/TCF-dependent promoter; 2) Determine sites of GFP transgene expression during mammary gland development, involution and remodeling. The ability to identify and isolate stem cells would simplify their characterization as well as the study of factors necessary for proliferation, differentiation, and susceptibility to transformation.

Body

The Statement of Work for this research had the following tasks and time line: Task 1) Generate transgenic mouse lines expressing GFP from a LEF/TCF-dependent promoter; and, Task 2) Determine sites of GFP transgene expression during mammary gland development, involution and remodeling.

Months 0-1: generate LEF/TCF-GFP reporter gene and validate expression in cell line (task 1).

Months 1-6: generate transgenic mouse founder lines expressing Lef-GFP (task 1).

Months 6-12: generate F1 transgenic lines and characterize GFP expression (complete task 1 and 2).

Task 1 was completed, but for reasons described below, I failed to complete Task2. I generated a construct with GFP driven by a LEF/TCF promoter, which displayed Wnt-dependent activation in cells in culture. This construct was digested with restriction enzymes to remove bacterial sequences, gel isolated, and injected into the pronucleus of fertilized mouse eggs. The injected eggs were re-implanted into psuedo-pregnant females, and the subsequent pups were analyzed for the presence of the transgene by PCR of genomic tail DNA using primers that specifically amplify a portion of the GFP gene. Initially, the generation of putative transgenic mice was performed by the Core Facility at The University of Maryland Institute of Human Virology, however, two attempts failed to yield any transgenics (thus losing approximately 6 months of time). Subsequent injections were performed by the Transgenic Core Facility at the University of Maryland School of Medicine. PCR analysis of several litters yielded 4 Lef-GFP transgenic mice.

Task 2: As of the end of this project, two of the founders were successfully bred, and passed the transgene to the F1 generation, thus resulting in a least 2 lines of Lef-GFP mice. Task 2 was not completed for several reasons. First, due to the initial failure to generate transgenic founders, I did not obtain the F1 transgenics until month 10 of the

project, thus leaving minimal time to complete Task 2. Second, the University of Maryland School of Pharmacy terminated my employment at month 11 of the project due to my inability to obtain further salary support. Since I still had supply money on this grant, I asked for a temporary position as a Visiting Professor (with no salary) so that I could attempt to complete Task 2, but was denied this request. At present, I am attempting to transfer the mice to a colleague at a different University to complete their characterization.

Reportable Outcomes

We have generated 2 transgenic mouse lines with the Lef-GFP construct.

This work formed the basis of an NIH R21 grant proposal.

This work gave me experience to apply for a position as the Head of a Stem Cell Core Facility.

Key Research Accomplishments

Generated at least 2 lines of transgenic mice expressing the Lef-GFP transgene.

Conclusions

I have generated at least 2 lines of transgenic mice that express the GFP reporter gene from a Lef-dependent promoter. Further work is needed to characterize the sites of expression of the transgene and determine if these mice will be useful for isolating mammary gland stem cells, as well as stem cells in other organs and tissues.

"So what section": Should the Lef-GFP transgene be expressed in stem cells of the mammary gland, this would allow for their isolation by fluorescence activated cell sorting. The isolated stem cells would allow detailed examination of the factors required for stem cell maintenance and differentiation. As it is believed that mammary gland stem cells are the primary target for cancer development, these studies could lead to potential new therapies for breast cancer, including new targets for drug development.

References

None.

Appendices

None.