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TITLE: Role of c-myb in Breast Development and Cancer

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In normal breast development, oncogenes and growth suppressor genes affect the cellular differentiation and growth rate and in tumorigenic state, they dictate metastatic pattern and response to treatment. Therefore, it is necessary to examine the role these genes play in normal and tumor cell growth and differentiation. c-myb belongs to the myb gene family which code for nuclear proteins that bind DNA in a sequence-specific manner and function as regulators of transcription (1). Recent evidence suggests a role for c-myb in breast development and breast cancer. c-myb is highly expressed in all estrogen receptor positive (ER+) breast tumors (2); and our in situ hybridization studies show that c-myb is expressed at high levels in ductal cells of virgin and pregnant breast tissue but is down-regulated in lactating breast tissue. These observations suggest that c-Myb might play a critical role in estrogen-mediated ductal cell proliferation. To address the role of c-myb in mammary development we propose to develop c-myb mutant mice where the expression of this gene is interrupted specifically in the mammary gland using the Cre:lox system. In addition, we propose to examine whether deregulated expression or structural alterations in c-myb gene leads to the genesis of breast tumors.
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Introduction

The development of breast cancer is a multistage process involving alterations in tumor suppressor genes and oncogenes. Overexpression of c-myb has been reported in human mammary carcinomas (1). c-myb belongs to the myb gene family which also includes A-myb and B-myb (2). All three members of this family code for nuclear proteins that bind DNA in a sequence-specific manner and function as regulators of transcription (2).

c-myb is predominantly expressed in hematopoietic cells and its essential role for the proliferative potential of these cells has been well established (2). Homozygous null c-myb mutant mice die in utero due to defects in fetal hepatic hematopoiesis (2,3). However, the role of c-myb in breast development and breast cancer is beginning to emerge only recently. The first evidence that implicated a role for c-myb in breast tumors came from the observation that this gene is highly expressed in all estrogen receptor positive (ER+) breast tumors (1). In addition, expression of a dominant negative mutant of c-myb in ER+ breast carcinomas was found to result in their growth arrest and loss of tumorigenicity (our unpublished observations). To determine whether c-myb gene plays a role in breast development, we examined the pattern of expression of this gene in breast tissues derived from virgin, pregnant and lactating mice. In situ hybridization studies show that c-myb is expressed at high levels in ductal cells derived from virgin and pregnant breast tissues but is down-regulated in lactating breast tissue. This observation combined with the observation that c-myb is highly expressed in ER+ breast tumor cells suggests that this gene might play a critical role in estrogen-mediated ductal cell proliferation.

Body

My application has two objectives:
1. To study the effects of c-myb gene deletion on breast development using the Cre-lox system.
2. To study the effects of oncogenic activation of c-myb gene on breast tumor formation.

Figure 1: Verification of homologous recombination at the c-myb locus, 11.5kb(wt), 12.6kb(recombinant).
When I began this work as mentioned in my original application, the targeting vector had already been constructed. In addition, potential recombinant ES cell clones that carry the conditionally targeted c-myb allele were also made available to me. Over the course of this year, I isolated DNA from 6 of these potential recombinant ES clones, digested with StuI, and analyzed by Southern blotting using a probe for exon 9A to verify the recombinant nature of these cells. As shown in figure 1, 4 of the 6 that I analyzed were true recombinant clones. I took these four clones and transiently transfected Cre into them via electroporation of 10μg of supercoiled pPGK-Cre-bpA as previously described (3,4). Subsequently, these cells were plated on gelatin-coated, irradiated-Mef culture plates. Four days after transfection, ES cells were selected with 2μM Ganciclovir for 9 days. This process yielded 35 subclones of which none are type II conditional c-myb mutants as analyzed by Southern blotting using a c-myb specific probe upstream of exon 2 after digestion with BamHI (see figure 2). After discussing this negative results with another member of the laboratory who has extensive experience with getting chimeric mice with his recombinant ES cell clones, the consensus was that the amount of supercoiled pPGK-Cre-bpA I used was probably not high enough.

Figure 2: Southern screening for type II conditional c-myb mutant ES clones, 11.5kb(wt), 6kb(type II conditional c-myb).

I am in the process of repeating the expression of transient Cre in these recombinant ES clones. In addition, I have also included 7 other potential recombinant ES clones that I have verified for homologous recombination in the c-myb locus. I have transfected 20μg and 50μg of supercoiled
pPGK-Cre-bpA into all of these recombinant ES clones. They are currently on their fifth day of Ganciclovir selection.

Though I have not proposed this in the original application, I have constructed a dominant acting c-Myb (cMET) construct under the MMTV promoter in case I am unable to get any type II conditional c-myb subclones or chimeric mice. I took the DNA binding domain (DNABD) of mouse c-Myb (aa 1 to 201), fused the DNABD to the Engrailed repressor domain (aa 2 to 298) via fusion PCR, and put a T7 tag at the C-terminal region. The creation of this type of dominant acting construct has been described (5,6,7). In addition, Weston and co-workers have used this approach to produce a transgenic mouse carrying a dominant acting c-Myb transgene under the T-cell specific promoter (8). I am employing this same approach to construct a dominant acting c-Myb whose expression is limited to the mammary tissue. This construct has been sub-cloned into the Smal site of pMAM and sequenced. Currently, I am trying to express this construct in culture cells before setting up to inject this MMTV-cMET-pA into pronuclei staged eggs.

I have also constructed an activated form of c-Myb, termed t-Myb, as formerly described by our lab (9). The t-Myb contains only the DNA binding domain and the transactivation domain of c-Myb. The C-terminal negative regulatory domain of c-Myb was omitted in t-Myb. Our lab and others have shown that t-Myb has greater transactivation potential than c-Myb (9,10). This t-Myb construct was tagged with HA epitope at the 5' terminus and sub-cloned into the Smal site of pMAM vector, which is driven by the MMTV promoter. The complete t-Myb insert was sequenced. Currently, I am attempting to express this construct in culture cells. After verifying for expression, the t-Myb insert, MMTV promoter, and polyadenylation signal (pA) will be excised via digestion with HindIII and BamHI. The MMTV-t-Myb-pA DNA construct will then be injected into pronuclei staged egg and transferred into pseudopregnant mice.

**Key Research Accomplishments**

- Verify that we have a total of 11 recombinant c-myb ES clones.
- Construct a dominant acting c-Myb construct under the MMTV promoter.
- Construct an activated form of c-Myb, termed t-Myb, under the MMTV promoter.

**Reportable Outcomes**

None

**Conclusions**
We have verified that we have a total of 11 recombinant c-Myb ES clones that have the targeting construct. We were unable to obtain any type II conditional c-myb ES clones in our first attempt. This may be due to the fact that the expression of cre is not high enough. Therefore, we are repeating transient Cre expression with higher amount of supercoiled pPGK-Cre-bpA. We have constructed a dominant acting c-Myb construct under the MMTV promoter which will provide insight into the importance of c-Myb in regulating its target genes. Lastly, we have constructed an activated form of c-Myb, termed t-Myb, under the MMTV promoter. Together these mice once generated will help in establishing a role of c-Myb in breast development and tumor genesis.

References


Appendices

None