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In order to develop a mouse model system that allows for rapid assessment of genetic lesions involved in breast tumor development, we are adapting a somatic gene transfer system based on avian leukosis virus A (ALV-A) expression vectors and TVA, the receptor for ALV-A. Transgenic mice have been generated that target *tv-a* to the mammary epithelial cells using MMTV and WAP promoters. Mammary epithelial cells from these transgenic mice are susceptible to infection both in vivo and in vitro. Low-grade carcinomas can be induced to progress to higher-grade tumors using viruses that express potent oncogenes. The mouse lines generated will be valuable research tools for gene delivery using the ALV-TVA system.

PTEN is a tumor suppressor gene mutated in many neoplasias including breast cancer. It encodes a lipid phosphatase that prevents the activation of Akt. Breeding of a *Pten* mutant allele into female mice predisposed to mammary oncogenesis, due to transgenic expression of MMTV-*Wnt-1*, led to more rapid formation of mammary tumors. This finding confirmed the role of *Pten* in breast tumorigenesis.

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INTRODUCTION

A large number of genetic lesions, both somatic and hereditary, have been identified in human breast cancer. These include *p53*, *BRCA1,* and *BRCA2*, *PTEN/MMAC1*, *neu/ErbB2/HER2*, *ErbB1/EGFR*, *PRAD-1/cyclin D1*, *Mdm2*, and *c-myc*. The molecular events responsible for specific steps in the initiation and progression of breast cancer are unclear. Many animal models have been created, but the systems are limited in the number of genes that can be introduced and examined. My research is designed to use a viral gene transfer system (Federspiel et al., 1994) to overcome some of the limitations. This system is based on the ability of avian leucosis virus subgroup A (ALV-A) to transduce TVA+ mammalian cells without production of viral (reviewed by Fisher et al., 1999). Consequently, after generating a single transgenic line expressing *tv-a* in a target tissue, individual and combinatorial effects of any oncogenic genes can be examined using ALV-A as a delivery vehicle. This system has been demonstrated to work in modeling gliomas (Holland et al., 2000; Holland et al., 1998; Holland & Varmus, 1998). I have adapted this approach to the mammary gland to study the effects of several breast cancer genes in mammary oncogenesis.

BODY

Task 1. Establish mammary-specific gene transfer methods (months 1-6).

Using a polyclonal antibody against TVA in immunohistochemistry, I have selected, among over twenty founder lines, four transgenic lines that express *tv-a* from mammary-targeted promoters (MMTV and WAP). Cultured mammary epithelial cells prepared from one MMTV-*tv-a* line (MA) can be infected with RCAS (a derivative of ALV-A) encoding alkaline phosphatase (RCAS-*AP*). The percentages of infected cells can be significantly increased (from 5-10% to 30%) by the use of concentrated viruses and by multiple infections. Preliminary results show that these cells can be doubly infected, as we anticipated. Direct injection of RCAS-*AP* into mammary glands in mid-pregnant transgenic mice resulted in AP staining in some ducts, suggesting that these cells are susceptible to infection in vivo.

Task 2. Introduce protooncogenes/oncogenes and dominant-negative TSGs into mammary glands in an effort to produce tumors (months 3-24).

I have made several RCAS viruses to express oncogenes and dominant negative tumor suppressor genes that are implicated in breast cancer. Among the viruses generated are RCAS expressing a mutant form of *HER2/neu*, *c-myc*, *Akt*, *cyclin D*1, a dominant-negative mutant of *p53*, *E-cadherin*, and *TGF-beta receptor 1*. Infection of two MMTV-*tv-a* mice with RCAS viruses encoding Wnt-1, cyclin D1, and FGF-3 led to hyperplasia and dysplasia in seven months, while two non-transgenic control mice did not display any ductal lesions. Experiments are underway to generate tumors by infecting proliferating ducts with viruses encoding more potent oncogenes such as polyoma middle T antigen or HER2/neu.

Task 3. Generate a mouse line carrying a floxed *Brca2* allele in collaboration with Anthony Wynshaw-Boris (months 1-12).

A targeting construct was made with both negative and positive selection markers. Transformation of this construct into bacteria expressing the gene encoding the Cre recombinase was found to yield a smaller plasmid,

with the size expected for the correct excision of the intervening DNA flanked by the loxP sites. Transfection of this construct into ES cells, followed by positive and negative selections, yielded 210 stable colonies. However, assays using Southern hybridization and PCR screening did not detect any colonies that had undergone homologous recombination. Since several other laboratories have already generated targeted mice carrying floxed *Brca-2*, I have discontinued further efforts to generate this line of animals.

Task 4. Delete floxed TSG in mammary glands at targeted times in hopes of generating tumors (months 12-24).

I have obtained a Cre reporter line—ZAP (Lobe et al., 1999), which expresses *LacZ* or *AP* depending upon the absence or presence of Cre, and have crossed it into the MA line. Mammary epithelial cells prepared from this line will be infected with RCAS-*Cre* to determine the deletion efficiency of the floxed *LacZ*.

I have imported mice carrying floxed *Brca-1* (Xu et al., 1999) and have bred the floxed allele into the MA line. The resulting *tv-a* TG, $Brca^{fl/fl}$ will be used for infections with RCAS-*Cre* in combination with oncongenic viruses such as RCAS-DN *p53*, RCAS-*neu* and RCAS-*c-myc*.

Task 5. Express protooncogenes/oncogenes and inactivate floxed TSGs in the mammary glands in order to generate tumors (months 24-36).

This section represents future work.

Task 6. Characterize tumors generated in the course of this study (months 24-36).

This section represents future work.

A new direction of this research

Human breast cancer is generally believed to progress from atypical hyperplasia to ductal carcinoma in situ, invasive carcinoma and metastasis. The majority of invasive human breast cancers are high-grade tumors. Distal metastasis occurs frequently and is usually the cause of mortality. Therefore, effective therapies are needed to intervene in cancer progression and to prevent or treat metastasis. To achieve these goals, it is essential to determine the underlying molecular events. However, mammary carcinomas arising in most transgenic mouse lines are less aggressive compared to human breast cancer and do not metastasize. I have started to use the tv-a lines created above to test various genes involved in cancer progression. MMTV-*Wnt-1* transgenic mice, which develop low-grade mammary tumors that rarely metastasize, have been bred to the MMTV-*tv-a* line to generate bi-transgenic mice. Candidate genes have been delivered to the developing tumors to determine if they could provoke tumor advancement. Initial results show that expression of HER2/neu and polyoma middle T antigen can lead to higher-grade tumors. Other candidate genes to be tested include genes encoding members of the TGF beta pathway (Bandyopadhyay et al., 1999; Yin et al., 1999), dominant-negative E-cadherin, certain chemokine receptors (Muller et al., 2001), and L-selectin (Qian et al., 2001). A refined version of this model system could be an ideal approach to test newly identified genes implicated in cancer progression and to dissect pathways involved in tumor invasion and metastasis. It could also be useful for animal testing of mechanism-based therapeutic drugs in both treatment and prevention.

Other achievements not proposed in the approved proposal

PTEN encodes a phosphatase that removes the D3 phosphate of phosphatidylinositol (3, 4, 5)-triphosphate, leading to the inactivation of AKT, a serine/threonine kinase that promotes survival and suppresses apoptosis. *Pten* is mutated in many human cancer types, including breast cancer. In mice, inactivation of *Pten* predisposes to multiple neoplasias, but mammary adenocarcinomas are infrequently observed (Di Cristofano et al., 1998; Podsypanina et al., 1999; Stambolic et al., 2000; Suzuki et al., 1998). I have generated a mouse model in which the role of *Pten* can be studied in breast cancer by crossing *Pten* heterozygous mice to MMTV-*Wnt-1* transgenic mice that routinely develop mammary carcinomas (Li et al., 2001). Female *Wnt-1* transgenics that were heterozygous for *Pten* developed mammary tumors at an accelerated rate compared to those with the wild type *Pten* alleles. In most tumors arising in Pten heterozygotes, the Pten wild-type allele was lost, suggesting that cells that lack *Pten* function have a growth advantage over cells retaining a wild type allele. Loss of *Pten* is accompanied by increased levels of phosphorylated AKT. This animal model may be useful in testing radiation and chemical therapies against tumors carrying a *Pten* mutant.

KEY RESEARCH ACCOMPLISHMENTS

1. I have created transgenic lines expressing *tv-a* from the MMTV promoter
2. I have created transgenic lines expressing *tv-a* from the WAP promoter
3. I have shown that mammary epithelial cells from MMTV-*tv-a* mice can been infected by avian leucosis viruses.
4. I have created ALV viruses expressing genes encoding HER2/neu, c-myc, Akt, cyclin D1, and polyoma middle T antigen.
5. HER2/neu expression can induce the advancement of a low-grade tumor into a more aggressive carcinoma.
6. Pten deficiency accelerates the onset of tumor formation in Wtn-1 transgenic mice.

REPORTABLE OUTCOMES

1. *tv-a* transgenic lines that express TVA in the mammary epithelial cells.
2. ALV viruses expressing genes HER2/neu, myc, Akt, cyclin D1, and polyoma middle T antigen.
3. Li, Y., Podsypanina, K., Liu, X., Crane, A., Tan, L. K., Parsons, R., and Varmus, H. E. Deficiency of Pten accelerates mammary oncogenesis in MMTV-Wnt-1 transgenic mice, BMC Mol Biol. *2:* 2, 2001.

CONCLUSIONS

I have generated transgenic mice expressing *tv-a* from mammary-specific promoters. The mammary epithelial cells from these mice can be infected with RCAS viral vectors. This model may be useful for studying molecular intearctions and cancer progression in mammary tumorigenesis.

I have determined that inactivation of Pten enhances mammary tumor development in mice that are predisposed to breast carcinomas, confirming its role in human breast oncogenesis.

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