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14. ABSTRACT Our objective is to develop a rodent model for human DCIS and LCIS in which lesions of diverse phenotypes can be induced and characterized as to their malignant potential that could be characterized in a linear manner. The majority of mammary cancers induced in rats by carcinogen treatment are ovarian hormone dependent for their growth. We tested whether treatment of ovariectomized rats with growth factors during carcinogen treatment would result in a higher percentage of hormone independent mammary cancers. Ovariectomized rats were treated with 10 ug per day of either EGF or IGF-1. On the fourth day of treatment the rats were treated with the carcinogen N-methyl-N-nitrosourea. The rats were treated for an additional 3 days with growth factor and they were then treated with either estradiol, progesterone, or estradiol plus progesterone to promote mammary carcinogenesis. The resultant mammary cancers were tested for the expression of estrogen receptor and progesterone receptor to determine their hormone dependent or independent phenotype.					
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Introduction

Our goals are to develop methods for the induction of a large number of ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) lesions with different phenotypes, be able to detect the lesions in situ and characterize the lesions as to their cancerous potential, hormone dependence or independence, and genetic changes. DCIS and LCIS are intraductal and intralobular hyperplasias. Proliferation of these cells is a prerequisite to carcinoma. However, intraductal proliferation with the exception of the terminal end bud occurring in peripubertal rats is extremely rare and has not been found in the terminal ductal structures during development or under experimental conditions. Our laboratory has made the novel finding that treatment of rats by infusing the mammary ducts with a combination of epidermal growth factor, cortisol, and cholera toxin causes extensive intraductal proliferation in the terminal ducts with a proliferation labeling index as high as 75% (1,2). Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (FGF 7) that is secreted by stromal cells and acts on epithelial cells. Treatment of intact rats with KGF causes massive intraductal hyperplasias (3). KGF can cause ductal growth and intraductal hyperplasias in ovariectomized mice. Concomitant treatment with estradiol and progesterone plus KGF increases intraductal hyperplasias. The intraductal hyperplasias regress after withdrawal of the mitogenic stimulus. We think that it should be possible to induce intraductal/intraalveolar hyperplasias by a variety of means and then treat with different chemical carcinogens to cause a large number of immortalized transformed phenotypes resembling DCIS and LCIS (4,5). These unique mitogens that cause intraductal proliferation have not been used, to our knowledge, in combination with ductal or alveolar mitogens in attempts to develop DCIS and LCIS. We believe that these treatments should result in an expanded pool of target cells for DCIS and LCIS which can then be transformed to preneoplastic and neoplastic states with well known mammary carcinogens such as N-methyl-N-nitrosourea (4), N-ethyl-nitrosourea, dimethylbenz(a)anthracene, or radiation.

Body

Task 1. Development of methods for the induction of DCIS and LCIS in inbred Lewis rats (months 1-12).

- a) Determine which hormones or growth factors or combinations induce intraductal or intralobular hyperplasia (3 rats/treatment).
- b) Quantitate proliferation by immunocytochemistry and confirm intraductal or intralobular hyperplasia by histology.

Task 2. Characterization of lesions as to their neoplastic potential and hormone dependency (months 12-36).

- a) Select lesions for expansion by serial transplantation based on their ability to proliferate and their neoplastic potential. Not started.
- b) Characterize resultant outgrowths and cancers as to their ovarian hormone dependence.

Approximately 80% of the mammary cancers induced in virgin rats by injection with the carcinogen N-methyl-N-nitrosourea (MNU) are dependent on the presence of ovaries for their

growth and immunohistochemistry indicates that the estrogen receptor (ER) and the progesterone receptor (PR) are highly expressed in the cancer cells. We tested whether ovariectomized rats challenged with growth factors at the time of carcinogen treatment would produce a higher percent of hormone independent mammary cancers.

Four-week-old female rats were divided into the following groups: 1. 18 rats left intact to serve as controls. The remaining 109 rats were ovariectomized. The completeness of ovariectomy was confirmed two weeks later by vaginal smear cytology. Three weeks later the ovariectomized rats received the following treatments: Group 2, no treatment. Group 3, 10 ug of EGF administered daily for one week by subcutaneous implant with a mini-osmotic pump. Group 4, 10 ug of IGF-1 administered daily for one week by pump. Four days after the beginning of treatment with growth factors the rats were treated with a single injection of the carcinogen MNU. Three days after MNU treatment, the pumps were removed and each group of rats either received no further treatment or was implanted with silastic capsules containing 20 ug of estradiol, 30 mg of progesterone, or 20 ug of estradiol plus 30 mg of progesterone to promote mammary carcinogenesis.

Approximately six months after carcinogen treatment. 100 % of the intact control rats developed mammary cancers. There were few mammary cancers in the growth factor treated groups not treated or treated with estradiol and progesterone for promotion. The mammary cancer incidence was 10.3% in the ovariectomized control rats, 13.3% in the EGF treated group and 21.4% in the IGF-1 treated group. Immunocytochemical analysis of the mammary cancers from the intact controls determined that ER and PR were highly expressed in the cancer cells, 87.2% of the mammary cancers were ER positive, 91.5% of the cancers were PR positive, 12.7% were ER negative and 8.5% were PR negative. 66.7% of the mammary cancers were ER positive and 66.7% were PR positive, 33.3% were ER negative and 33.3% were PR negative in the ovariectomized control rats. The EGF treated group had 55.6% ER positive, 66.7% PR positive, 44.4% ER negative and 33.3% PR negative mammary cancers. The IGF-1 treated group had 55.6% ER positive, 55.6% PR positive, 44.4% ER negative and 44.4% PR negative mammary cancers.

Previously, we have found that infusion of growth factors directly into the mammary nipple results in rapid proliferation of the mammary epithelium in ovariectomized rats and when treated with carcinogen up to 75% of the animals develop mammary cancers.

Key Research Accomplishments

- Previously demonstrated that infusion of hormones or growth factors results in rapid proliferation of the mammary epithelium within 72 hours
- Infusion of IGF-1 or EGF into ovariectomized rats results in the induction of intraductal proliferations.
- We are testing whether ovariectomized rats treated with growth factors at the time of carcinogen administration produce a higher percent of hormone independent mammary cancers.

Reportable Outcomes

None

Conclusions

Taken together, these results indicate that proliferation of the mammary epithelium can be induced quickly by infusion of mammogenic hormones and growth factors. The pattern of proliferation varies with the hormone or growth factor used. The induced proliferations can be neoplastically transformed with a chemical carcinogen. These findings suggest that it should be feasible to induce proliferation of the mammary epithelium with different agents and neoplastically transform them resulting in lesions with different morphologies and different phenotypes and genotypes. In the next grant period, it is our plan to induce proliferation of the mammary epithelium with different mammogenic hormones and growth factors, identify transformed lesions in situ, and transplant them to syngeneic hosts and develop stable lines with different morphologies and neoplastic potentials.

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