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

This application should be considered in the context of the competing theories of carcinogenesis. The first is *the somatic mutation theory* which is based on two main premises: the first, claims that the default state of cells in metazoa is *quiescence*, and the second posits that cancer is the result of the multistage process where successive mutations accumulate in a single target cell. Much was learned about genes expression under a variety of conditions. However, this aggressive effort failed to provide either an explanation for carcinogenesis or a rationale for effective therapies. The second theory is the *tissue organization field theory of carcinogenesis.* This theory has adopted two basic premises: the first postulates that proliferation is the default state of all cells (prokaryotes to metazoa), and the second states that tissues (stroma or epithelium, or both) are the targets of carcinogens.

The first aim tests whether the primary target of the carcinogenic agent nitrosometylurea is the epithelium, the stroma or both. Recombinants will be made where vehicle-treated stromal cells are recombined with epithelial cells from vehicle-treated and carcinogen-treated animals, and stromal cells from carcinogen-treated mammary fat pads are recombined with epithelial cells from vehicle-treated and carcinogen-treated animals. The second aim assesses histoarchitecture changes in the mammary gland in the tissue recombinants developed in Aim #1. Finally, the third aim documents molecular changes in epithelial and stromal pericellular matrices.

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INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The research project has three specific aims. The first is to determine which tissue is the target of the chemical carcinogen N-nitrosomethylurea in the rat mammary gland. This aim was scheduled to occupy the first 18 months of support. The second aim includes screening and counting lesions and performing morphometric data analysis of whole mounts (branching pattern, relative abundance of the different ductal and alveolar structures). These studies are aimed at identifying the changes occurring between the time of exposure and the appearance of neoplasias. We will also analyze BrdU incorporation, and identify changes in components of the extracellular matrix. Finally, during years two and three we will begin to test the specific roles of hyaluronan and emmprin, two molecules that are enriched in tumors and involved in tumor-stromal cell interactions as mediators of neoplastic initiation and progression.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement Of Work.

We anticipated that the first task of our research project would require 18 months to be completed. We are now at the end of the first 12 months and we have accomplished the following:

a) We have standardized the fat pad clearing procedure of 21-day-old rats. We have verified that 5 months after "clearing" the empty fat pads remained empty of epithelial cells showing the surgery to be successful. Moreover, inspection of whole-mounts of the removed ductal tree allows us to assess prior to cell injection whether the pad has been cleared effectively, and consequently, discard animals in which the procedure was incomplete. In addition, we injected 50,000 mammary epithelial cells (cultured in matrigel covered plastic flasks) into the cleared fat pads of 50 day-old animals to evaluate the length of time necessary for these cells to repopulate the "cleared" fat pads and the efficiency (% success) of these cell inoculations. We have noted that it takes over 30 days for the formation of a noticeable ductal tree. By 150 days, 1/3 of the "cleared" fat pad is covered with the tree-like epithelial growth. We used a set of 10 animals to assess the rate of success of epithelial cell takes. In this small set 60% of the injections developed ductal trees and 40% remained empty.

b) We also determined that the optimal number of animals that could be processed simultaneously is 20. Consequently, the experiment proposed, consisting of 20 animals per group, is being performed in batches of 20 animals, containing 5 animals in each of the four proposed recombinants (NMU-treated cleared fat pads plus vehicle-treated epithelial cells, NMU-treated cleared fat pads plus

NMU-treated epithelial cells, vehicle-treated fat pads plus vehicle-treated epithelial cells and vehicle-treated fat pads plus NMU-treated epithelial cells). We have processed three of these 20-animal groups. Rats whose mammary epithelial cells were "cleared" at 21 days of age have been injected with NMU or vehicle as described in the original application (at 55 days of age). Subsequently (five days later), these rats were inoculated with 50,000 cultured mammary epithelial cells, treated with either NMU or vehicle. Tumors were expected to develop in rats whose stroma was exposed to NMU, regardless of whether or not the injected mammary cells grown in culture were exposed to the carcinogen. For unbiased evaluation, groups of rats are handled blindly by personnel who determine the presence of tumors in mammary glands. Tumors have been felt in some rats while other animals show no palpable tumors. Given the ongoing nature of this experiment, we have not yet broken the blind code. When tumors reach 0.5 cm in diameter, they are excised leaving the remaining mammary gland tissue to develop additional lesions. The experiment is terminated 9 months after recombination. In addition, 2 groups of intact animals treated with vehicle (negative controls) or NMU (positive controls) are processed simultaneously with each recombinant batch. The excised tumors were assessed by histology. They were adenocarcinomas.

c) We are now in the process of collecting mammary glands of the different groups identified in the Experimental Design of our application (Groups 1 to 6). We are optimizing the protocols to characterize emmprin expression. Emmprin is a cell surface glycoprotein of the Ig superfamily that is up-regulated in malignant cancer cells, including mammary carcinoma *in vivo*. We have already collected data on patterns of expression of this marker in rats belonging to the above-mentioned groups. However, no comprehensive data are yet available from this analysis. We anticipate that these data will be available and evaluated in the next months; a comprehensive report will be presented at the end of the second year of support. In addition, we are starting to develop a protocol for the manipulation of hyaluronan expression. This will continue to be developed in the second and third year of support.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

• We have verified that the clearing procedure is effective and that cleared mammary glands remain free of epithelial cells when analyzed 5

months after clearing. Moreover, inspection of the removed ductal tree allows discarding animals in which the clearing was incomplete (i.e., if the margins had ductal tissue). This ensures that no false positives will be produced (i.e., animals whose epithelial remnants will be exposed to NMU).

- We have measured the efficiency of the epithelial cell injection, which is 60%.

- Tumors were harvested from the recombinants; histological examination revealed adenocarcinomas both in the positive controls and in recombinants.
- At this point it is not known which recombinant groups developed tumors since the codes will not be broken until the experiment is completed. However, since animals with tumors in the inguinal glands (only the 4th and 5th pair were cleared and used for recombination) also developed tumors in the thoracic glands we are inferring that the animals exposed to NMU are the ones that are developing tumors (i.e, recombinants whose stroma was exposed to NMU).

REPORTABLE OUTCOMES:
No publishable data has been collected so far due to the fact that this grant is still in its data collecting stage. However, a presentation on the rationale for running this type of experiment aimed at testing the robustness of the competing theories of carcinogenesis (the somatic mutation theory versus the tissue organization field theory) was made last March 17 at an international meeting in Berder, France.

CONCLUSIONS:
No conclusions could be drawn from experiments not yet finished.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in Science, Military Medicine, etc.).
No publications have been submitted based on the data collected so far.

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.
Not applicable.