

RESEARCH PLAN

a. Specific Aims

The identification of anti-apoptotic signaling mechanisms has provided an experimental framework for understanding how pre-malignant and malignant cells can escape environmental signals that would normally initiate programmed cell death. The critical importance of identifying survival pathways relevant to specific cell types is highlighted by studies that have associated activation of individual anti-apoptotic pathways with treatment failure in some tumor cell types and not others (1). In mammary epithelial cells (MECs), we have recently defined a novel glucocorticoid receptor (GR)-mediated survival mechanism that is induced by prolonged exposure to physiological concentrations of glucocorticoid and inhibited by GR-specific antagonists (antiglucocorticoids) (2). GR antagonists have been shown previously to interfere with glucocorticoid function by a two step process: 1) competitive inhibition of glucocorticoid binding and 2) competition of the antagonist bound receptor with that of the glucocorticoid-bound receptor on DNA response elements within target gene promoters (3). Because active antiglucocorticoid compounds inhibit the transactivation (and possibly the repression) of GR-specific target genes and also block GR-mediated survival signaling, we hypothesize that the mechanism through which the GR induces survival requires the induction and/or repression of cell type-specific "survival genes" (4).

In order to identify downstream targets of GR-mediated survival signaling, we have used cDNA array analyses to identify genes that are specifically induced in MECs following GR activation but whose transactivation is blocked by concomitant treatment with the potent antiglucocorticoid RU486. One such gene, a homologue of the serine-threonine kinase Akt, is serum and glucocorticoid-inducible kinase (SGK). We have subsequently found that ectopic expression of SGK inhibits MEC apoptosis in cells deprived of growth factors, suggesting that SGK may be an important transcriptional target required for GR-mediated survival signaling. We are now identifying additional genes whose protein products may contribute to the mechanism of GR-mediated survival signaling in breast epithelium.

Social isolation of rodents has been demonstrated to significantly alter both long-term and short-term regulation of the hypothalamic-pituitary-adrenal (HPA) axis. McClintock and colleagues (see Project 1 and Preliminary Studies, this project) and others (5, 6) have shown that rats subjected to long-term social isolation respond to this chronic social stressor by decreasing their basal corticosterone levels. However, the isolated rat's response to acute stressors of daily living (noises, temporary restraint etc.) is intact and in fact the time it takes for corticosterone levels to return to baseline is significantly prolonged (see Project 1 and Fig. 16. this project). Thus, the net effect of social isolation is predicted to cause increased GR signaling in response to acute stressors, both because of long-term up regulation of GC receptors (due to decreased basal corticosterone levels) and high sustained levels of corticosterone caused by failure of the short-term negative feed-back system. With continued social isolation and accompanying hypervigilance, a pattern of repeated and prolonged exposure to corticosterone is maintained throughout adulthood. The phenotypic result is that female Sprague Dawley rats, while genetically predisposed to developing mammary cancer, do so at an alarmingly early age when socially isolated. **We hypothesize that one of the key physiological mechanisms underlying accelerated mammary carcinoma formation in socially isolated rats is chronic activation of a GR-mediated mammary epithelial cell survival signaling pathway.** This pathway is expected to contribute to the accumulation of malignant cells that otherwise would undergo apoptosis. Together with the higher levels of estrogenization found in isolated rats (see preliminary data, Project 1), chronic GR signaling and estrogen-mediated proliferation could accelerate tumor development dramatically.

Using measurements of GR signaling such as GR-mediated gene regulation (DNA array analysis) and upregulation of GR-induced gene products (immunohistochemistry), this hypothesis will be tested in two rodent models of mammary gland carcinoma: the Sprague-Dawley rat and the transgenic C3(1)-SV40 T antigen (Tag) mouse in which the SV40 large Tag oncogene is targeted to the mammary epithelium. We believe it is important to test the hypothesis in two well-characterized models of breast cancer, both of which have a large literature base and a wide group of active investigators as resources for interpreting the novel data that will be gained from this multidisciplinary project. Evidence of activation of the GR survival signaling pathway will be examined in tumors and normal mammary glands from both isolated and group-housed animals at multiple stages of mammary gland tumor development using large scale gene arrays,

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immunohistochemical and biochemical analyses of OR-regulated gene products. In addition, we hypothesize that since apoptosis is known to play a critical role in chemotherapy-induced cytotoxicity of breast cancers, social isolation and subsequent GR activation in mammary glands will inhibit the response to cytotoxic therapy. Finally, we will test the hypothesis that chemoprevention of breast cancers is made less effective in socially isolated, hypervigilant rodents due to the and the inhibition of apoptosis.

Specific Aims

The overall goal of this project is to test the hypothesis that social isolation results in accelerated and chemo-resistant mammary gland cancer through a mechanism that is associated with increased hypervigilance, a prolonged corticosterone response to acute stress and consequent GR activation of, target anti-apoptotic genes.

1. Does social isolation of rodents alter glucocorticoid receptor (OR) expression and/or GR-regulated signal transduction in normal and malignant mammary glands?
2. Does social isolation result in relative tumor resistance to chemotherapy?
3. Does social isolation decrease mammary gland susceptibility to chemoprevention?