CHAPTER VI
CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

Breast cancer is a generic term for the diseased states which arise from the transformation of mammary epithelial cells. These cells interact with other tissue cells, affecting nearby cells and being affected by nearby cells via a paracrine system. Growth factors, binding proteins, and other secreted substances are normally released from the cell surfaces to affect, in a coordinated fashion, neighboring cells. This is an important means by which homeostasis is maintained among the tissue cells. One suspected cause of cancer cell proliferation is the overexpression of IGF-I or IGF-II by the transformed epithelial cells. IGF-I/IGF-II is suspected to stimulate the receptors of the same cells which produced the growth factor via an autocrine system.

Ultimately our goal is to develop the means to prevent or slow the stimulation of the IGF-IR by IGF-I or IGF-II in order to prevent or slow cancer cell mitosis. Because of the close homology of the IGF-I molecule to the IGF-II molecule, information obtained for IGF-I can also be related to IGF-II. IGF-I, IGF-II, and insulin have all been shown to have close homology and to act on the IGF-IR and insulin receptor (IR) (Blakesley, *et al*., 1996). The IGF-IR and IR are typically expressed on different types of cells, and unlike the IGFs which are bound in complexes and unavailable in the bloodstream, insulin travels alone and is available for glucose uptake while still in the bloodstream. The IGFs become available to cells after dissociation from the bound complexes. This compartmental separation allows the IGFs and insulin to have the very different functions of mitosis and glucose uptake, although under experimental conditions, both the IGFs and insulin can function in both capacities with limitations (Jones and Clemmons, 1995, Schofield, 1992). For example, IGF-I may stimulate glucose uptake via the insulin receptor, but lack the ability to stimulate glucose oxidation.

The increased production of IGF-I in the autocrine SV40-IGF-I cell line induces changes in the cells besides higher levels of secreted IGF-I and a possible proliferative advantage over the paracrine MAC-T cell line (Romagnolo, et al., 1994). The SV40-IGF-I cell line also secretes higher levels of IGFBP-3 and has reduced expression of IGF-IR on the cell surface. It was noted during preparation of cells for the microphysiometer experiments that the SV40-IGF-I cell line proliferated more quickly than the MAC-T cell line under the same experimental conditions and in general seemed to crowd to higher numbers on the tissue culture plate. This reduction in growth inhibition may be characteristic of autocrine systems for IGF-I or IGF-II.

By understanding the contributions of extracellular factors such as IGFBP-3 and proteoglycan on IGF-I binding to the IGF-IR, we can gain an understanding of how modifying the concentrations of these factors can affect IGF-I binding and, consequently, stimulation of mitosis. Ultimately, concentrations of binding inhibitors may be found to slow or prevent IGF-I stimulation and subsequent rapid proliferation of cancerous cells without having a major impact on healthy normal cells. This would be very important for the development of pharmaceutical interventions in cancer therapy.

In this thesis we have optimized system parameters using the Cytosensor® Microphysiometer System for the SV40-IGF-I and MAC-T cell lines. The Cytosensor® Microphysiometer System detects the real-time rapid binding of IGF-I to the IGF-IR via the excretion of protons. MAC-T cells showed a somewhat lower response than SV40-IGF-I cells to IGF-I in initial experiments, but this may be due to increased sensitivity of the MAC-T cell line to environmental stress. In a later experiment for which cells were maintained at the test location, both lines had the same average % ECAR response to IGF-I.

The focus of this study after system optimization has been to compare IGF-I stimulation of the autocrine SV40-IGF-I cells and paracrine MAC-T cells and to investgate how stimulation of the IGF-IR may be slowed by the presence of IGFBP-3 in the SV40-IGF-I cells.

We found:

1.) IGF-I stimulates SV40-IGF-I in a dose dependent manner.

2.) IGFBP-3 likely inhibits IGF-I stimulation of SV40-IGF-I cells in a dose dependent manner. Preliminary studies suggest this to be so, but additional data is needed to verify this relation.

3.) IGFBP-3 does not show IGF-I independent stimulation or inhibition of proton production by the SV40-IGF-I cells in the rapid binding experiments. Data from 3 hr binding studies suggest IGFBP-3 may bind with high affinity to a small number of receptors on the SV40-IGF-I cell surface. Additional studies are necessary to verify this.

4.) IGF-I and R^3IGF-I show similar stimulation profiles with borderline significantly different % ECAR peak heights from stimulated SV40-IGF-I cells. Since R^3IGF-I is the IGF-I analogue with lower affinity for IGFBP-3, this suggests that binding of IGF-I to constitutive IGFBP-3 may not significantly inhibit IGF-I receptor binding under the rapid binding conditions. Larger sample sizes are needed to confirm this.

In addition, SV40-IGF-I cell secreted proteoglycan has been harvested from conditioned media and processed for use on the microphysiometer.

6.2 Future Work

Further characterization of the source of extracellular acidification, or the ECAR response on the microphysiometer, is needed to confirm the source of the proton efflux. Agents such as amiloride with known sites of action on the cellular pathways can be used to further confirm the source of the proton excretion. In addition to these agents used on other cell lines, stimulation by IGF-II and insulin with known affinities for the IGF-IR can be used to further characterize the ECAR response.

To compare autocrine and paracrine stimulation of the cell by IGF-I, the microphysiometer studies with IGF-I and IGFBP-3 performed with the SV40-IGF-I cells will be repeated with the MAC-T cells. Since we are also interested in the relative contributions of IGFBP-3 and cell secreted proteoglycan to the binding of IGF-I, we will investigate combinations of these agents on both cell lines. Mathematical modeling of this behavior could be valuable for the design of therapeutic interventions. We will look for combinations of IGFBP-3 and proteoglycan in concentrations which will inhibit IGF-I binding, and therefore inhibit mitogenesis. Future work will also characterize the proteoglycan of the MAC-T and SV40-IGF-I cell lines.

In addition to correlating the results of traditional binding studies to the results of rapid binding studies on the microphysiometer, cell proliferation studies using thymidine incorporation will be used to correlate the longer term growth to the binding results. Flow cytometry experiments will be conducted to further verify ligand binding to cell surface.

SV40-IGF-I cells show induced changes such as increased IGFBP-3 production and decreased number of IGF-IR receptors on the cell surface compared to the parental MAC-T cell line. To investigate the possible effect of components in SV40-IGF-I conditioned media on the MAC-T cells, MAC-T cells will be incubated in the SV40-IGF-I conditioned media and compared to control MAC-T and SV40-IGF-I cells for conditioned media induced changes. For additional comparison of paracrine and autocrine responses, MAC-T cells grown with exogenous IGF-I will be compared with SV40-IGF-I cells grown in plain media.

In conclusion, the combination of the optimization of the Cytosensor® Microphysiometer System and of the preliminary studies using the paracrine and autocrine mammary epithelial cell lines sets the groundwork for continuing studies for the quantitative comparison of these two cell systems. With quantitative studies of the spatial and temporal environments of mammary epithelial cells, cellular environments conducive to

cancerous mammary epithelial cell growth can be compared to cellular environments conducive to normal mammary epithelial cell growth. Armed with this additional quantitative information, interventions can be better planned and designed for more specific therapies in the fight against cancer.