The nucleus is the compartment within each cell that contains the genetic information directing how the cell grows and behaves. Although pathologists use an enlarged nucleus to diagnose cancer and determine what stage it has reached, we know very little about what causes large nuclear size or what the consequences are for the cancer patient. My lab studies the model organism *Xenopus* (African clawed frog). Similar systems regulate cell growth in humans and frogs. In fact, proteins from human cells often work in frog cells. *Xenopus* research has been important in studying cancer, as well as congenital heart disease, progeria, and Fanconi anemia, to name a few. We anticipate that discoveries about nuclear size control in *Xenopus* will translate to humans, producing important information for the cancer community.

We are using *Xenopus* embryos to understand how nuclear size is controlled during embryo development. In many ways, the uncontrolled growth of cancer is similar to rapid cell growth in developing embryos. In fact, cancer may arise from reactivation of embryonic growth programs in otherwise normal cells. Understanding nuclear size regulation in embryos will therefore inform cancer. To translate our findings in *Xenopus* to humans, we propose to directly alter nuclear size in cancer cells. To our knowledge for the first time, we will directly test if reducing the size of the nucleus slows cancer cell growth and metastatic potential. Our studies should shed light on how nuclear size contributes to cancer development and progression. Novel approaches to cancer diagnosis and treatment that target nuclear size will be suggested, and new cancer susceptibility factors associated with altered nuclear size could be identified to aid in prevention.

**Objectives**

The objective of this study is to use information we have gained from the *Xenopus* system about mechanisms of nuclear size control to test if reducing nuclear size in human cancer cells affects their growth properties. Importantly, these basic studies in cell biology should provide the necessary information to develop novel methods to control cancer.

**Material and Methods**

We previously identified a protein, Ntf2, which regulates nuclear size in *Xenopus*. Ntf2 plays a role in regulating nucleocytoplasmic transport. To test the effect of Ntf2 expression on nuclear size and cell growth in human cancer cells, we are using a well-established human prostate cancer cell line called LNCaP. We transfected an Ntf2 expression plasmid into LNCaP cells and used antibiotic selection (geneticin) to isolate several stable cell lines that overexpress Ntf2. The ectopically expressed Ntf2 was tagged with an mCherry fluorescent marker, allowing us to visualize Ntf2 expression in cells. We imaged nuclei in these stable cell lines using a DNA stain (Hoechst) to assess effects on nuclear size. We also measured the proliferation rates of these cell lines using a cell counter (Countess® from Life Technologies).

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Results and Discussion

We found that stable overexpression of Ntf2 in LNCaP cells led to a reduction in nuclear size (Figure 1). This is consistent with how Ntf2 regulates nuclear size in Xenopus. Strikingly, these cell lines with reduced nuclear sizes exhibited reduced cell proliferation rates (Figure 2). We also observed that cells overexpressing Ntf2 exhibited altered colony morphology suggestive of reduced cell spreading (Figure 1). These data indicate that reducing nuclear size in cancer cells may be sufficient to slow the growth rate of these cells. Future studies will address whether reducing nuclear size impacts other cancer characteristics, such as apoptosis (programmed cell death) and cell migration, and we will test if these stable cell lines have reduced tumorigenic potential in mice.

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