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Exploring Prostate Cancer Treatments Through Ciliogenesis

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Abstract

Cancer is a result of uncontrolled cell division. When normal cells get close to other cells they stop dividing, whereas cancer cells continue to divide on top of each other regardless of proximity, causing a tumor. Cancer is not one disease, but rather a collection of several different types of diseases that are all caused by uncontrolled cell growth. Prostate cancer is the second leading deadly cancer among men. Furthermore, there have been clear links between African American men and an increase of both diagnosis and mortality. The aim of this study is to identify a possible alternative treatment that can help various men across the world, but specifically African American men. The approach used in this experiment was to force the prostate cancer cells into G0 phase with media starvation and or pharmaceutical drugs, which will make the cells seize from multiplying. Cells that are in G0 phase are not actively dividing and will grow primary cilia. The hypothesis is that by forcing prostate cancer cells to grow primary cilia they will stop actively dividing. Furthermore, if this is achieved by pharmaceutical drugs future chemotherapeutic approaches may include placing beads of ciliogenic agents directly into the tumor to inhibit cell division and thus tumor growth. In this experiment the steroid dexamethasone and cell starvation were used to test the hypothesis. Both dexamethasone and cell starvation have shown some primary signs of inducing cilia. With prostate cancer affecting a large group of the United States population and specifically African American men, this treatment could change the approach to chemotherapy and alleviate this health disparity.

Keywords: Prostate Cancer, Cilia, Staining, Antibody

1. Introduction

Prostate cancer is not nearly discussed as frequently as other cancers like lung cancer or breast cancer, yet it is the second most deadly cancer to men.[1] According to the American Cancer Society 1 in 9 men will be diagnosed with prostate cancer in their lifetime.[2] Although the number of diagnoses is astronomical, the rate of survival is extremely promising, if the diagnosis is made prior to metastasis. The survival rate after five years is nearly 100 percent.[3] There is a disproportionate occurrence of prostate cancer in African American men and Caribbean men of African descent. In addition, African American men are twice as likely to die from prostate cancer as their white counterparts.[4] Despite the data on this health disparity, the research on chemotherapeutic modalities targeted to African American men with prostate cancer are limited.

The current treatment options for prostate cancer, such as surgery, external radiation, and brachytherapy, are usually restricted to younger patients while older patients, generally over the age of 70, are kept under close observation.[5] If new treatments are not developed, the mortality rate for this group will continue to increase. The overarching objective of this project is to further our understanding of prostate cancer cells on a molecular level and possibly develop treatment modalities.

Cancer is a result of uncontrolled cell division; when normal cells get close to other cells they stop dividing whereas cancer cells continue to divide on top of each other causing a tumor. Progression through the cell division cycle is

regulated by a series of checkpoint mechanisms. In the G1 phase, actively dividing cells must pass through the restriction checkpoint (R); cells that fail to pass this checkpoint become arrested in G1 or enter a quiescent phase known as G0. In G0 the centrioles transition to basal bodies and nucleate a primary cilium (in most cell types). The presence of a primary cilium is an indication that the cell is not actively dividing.[6] This primary cilium is a sensory organelle that allows the cell to communicate with the external environment. Many signaling pathways function through the primary cilium, most notably, the Hedgehog signaling pathway.[7]

The overarching goal of the Hendrickson lab in the Morehouse College Biology Department, is to further elucidate the role of primary cilia in diseases, and specifically in prostate cancer. There are two different types of cilia: motile and immotile. Motile cilia are used all over the body. They are used in the brain to help move the cerebral spinal fluid around. Cilia are an essential part of the respiratory system as well, lining trachea to assist keeping the airway clear, in combination with the mucus on the "Mucus Elevator" helping to keep the lungs clean. Additionally, cilia are essential in reproduction: the sperm tail is a single cilium that beats to allow the sperm to swim in the vaginal canal toward its goal of reaching the egg; in the female reproductive tract, cilia line the fallopian tubes and help the egg travel from the ovaries to the uterus. Cilia move in a circular direction resembling a lasso; flagella, a longer version of cilia, move in a sweeping direction back and forth. Immotile cilia do not move, as the name implies. Rather, they extend from the cell body and serve as an antenna to receive and process signals for the extracellular environment.[8] These signals include telling the cell when to divide and when not to divide. These immotile cilia are also called primary cilia and they are found on almost every type of epithelial cell in the human body. Cells that are actively dividing do not have primary cilia; rather, the centrioles transition into centrosomes from which the mitotic spindle is nucleated in the M phase. Since cancer cells are cells that are undergoing uncontrolled cell division, they do not have cilia. The hypothesis is that prostate cancers cells can be forced to enter G0 by inducing ciliogenesis.

The first step in this study was to establish that our experimental system, the prostate cancer cell line PC3, do not contain primary cilia and then determine whether ciliogenesis be induced in a manner similar to that of nutrient starvation by treating the cells with an existing pharmaceutical agent. In addition to the PC3 cells, we used HEK 293 cells as a control. Since Dexamethasone has been previously used to induce cilia in other cancer lines, it was used in conjunction with starvation, to induce ciliogenesis in the HEK 293 and PC3 cells.[9]

2. Methods

For this experiment the PC3 cell line and HEK 293 cells were used. The cells were thawed, grown to confluency, then split, which consisted of taking 40% of the cells and placing them aside and then using the remaining 60% of them on the six well plates containing sterile glass coverslips (Figure 1, 2). The PC3 cells, were grown according to the following: Column 1: DMEM + 10% growth serum, which is the standard amount of growth serum; Column 2: DMEM + 0.5% growth serum (starvation conditions); Column 3: DMEM only (no growth serum (Figure 1). The HEK cells were grown under the following conditions: DMEM + 10% growth serum; DMEM + 10% growth serum + Dexamethasone; DMEM + 1% growth serum; DMEM + 1% growth serum + Dexamethasone (Figure 2).

Next the cells were fixed in 2% paraformaldehyde in PHEM, followed by a 2-minute extraction with 0.1% Triton X- 100 in PHEM. The cells were then washed in 1X PBS then blocked with 1% bovine serum albumin (BSA) in TBST for one hour. Next, the cells were incubated with the primary antibodies (anti- acetylated α-tubulin, anti-ITF81, or anti-Arl13-b) overnight at 4°C. Thereafter, the cells were washed four times with 1% BSA/ TBST over about 30 minutes. The cells were then incubated with 1:2000 dilutions of Alexa 350-, 488-, 594-, 633-, or 680- conjugated anti mouse IgG or anti rabbit IgG secondary antibodies for 1 hour. The cells were then washed three times with 1% BSA/TBST for about 30 minutes. After the third wash, the cells were incubated with rhodamine, then DAPI for 30 minutes then washed with PBS, then dH_2O, before mounting. The stained cells were viewed using a fluorescent microscope, Zeiss Axio Imager A2; image capture and analysis were done using the Zen imaging software (Figure 3).

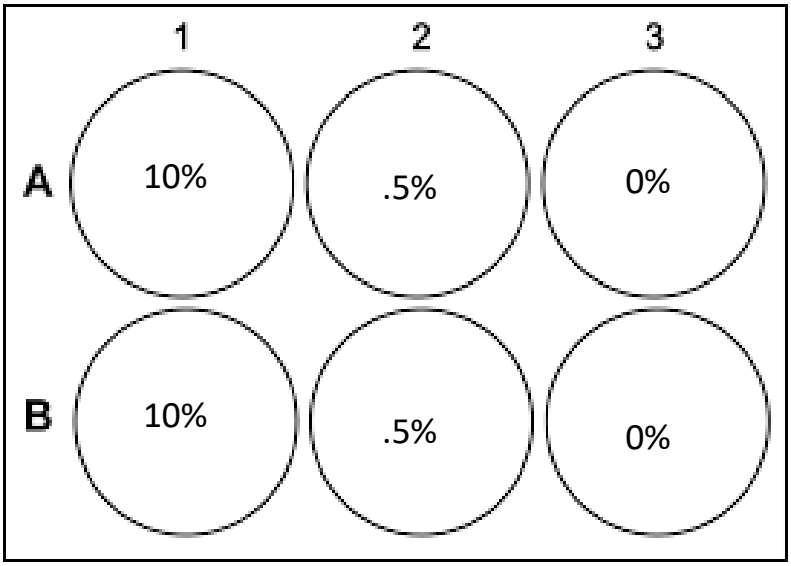

Figure 1. PC3 cells were grown in 6-well plates. The PC3 cells were grown in three different media conditions: DMEM plus10%, 0.5%, and 0% growth serum. Cells were grown for 48h then fixed and stained.

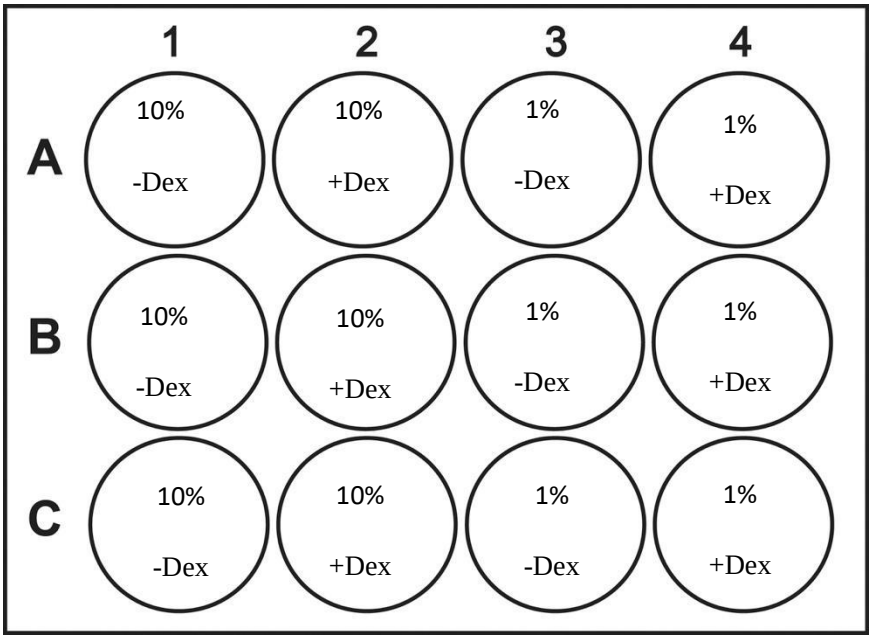

Figure 2. HEK 293 cells were grown in 6-well plates under varying conditions. This plate contained the HEK 293 cells with the four different conditions: DMEM 10% +Dex, 10% -Dex, 1% +Dex, 1% -Dex. Cells were grown for 48h, then fixed and stained.

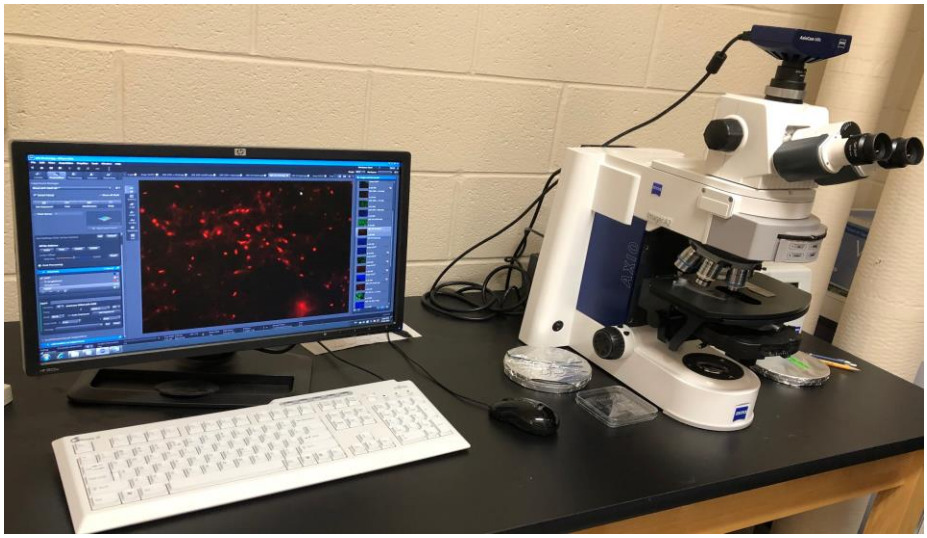

Figure 3. Fluorescent Microscope. This Zeiss Axio Imager A2 was used to visualize the stained cells. Images were captured and analyzed using Zen software.

3. Results

The overall goal of our research is to better understand the role of cilia in disease and development, with an eye towards developing additional treatment modalities for prostate cancer. This study in particular was designed to determine whether cells could be induced to grow cilia as a result of starvation and/or treatment with Dexamethasone. HEK 293 and PC3 cells were grown under normal conditions and under starvation conditions, both in the presence and absence of Dexamethasone. Multi-well plates were used to ensure that the growth conditions, minus the growth serum and Dexamethasone, were the same for all cells. Overall, the PC3 cells behaved the way that we hypothesized. As shown in Figure 4, the results from the first column, which contained the standard amount of growth serum 10%, were as follows: no cilia were seen as indicated by the lack of acetylated α-tubulin staining in the red channel; however, the cells appeared healthy as indicated by the presence of nuclei (DAPI staining). This was expected as cells would be actively dividing when sufficient levels of nutrients are present. Additionally, when observed by phase-contrast (not shown), the cultures appeared to be nearing confluency. In the second growth condition of 0.5% growth serum, there appeared to be a few cells with cilia (Figure 5). There were no noticeable changes in the DNA and actin staining of the 0.5% cells when compared to the 10% cells. There were also fewer cells in the second column, which represented starvation conditions, compared to the 10% column. Moreover, the cells appeared to be splotchy looking and more rigid, traits that are indicative of cancer cells. In the extreme starvation condition (0% growth serum), there were many distinct dots of acetylated α-tubulin staining, which can be interpreted as being indicative of primary cilia (Figure 6). Similar to the 0.5% column the actin and DNA appear to be unchanged. Under these conditions, there appeared to be an extreme reduction in cell growth and numbers and these cells also resemble cancer cells with their morphology.

Overall, the HEK cells behaved the way that we hypothesized, except for the 1% with Dexamethasone. The first column, which was grown in the 10% growth serum without Dexamethasone, results were as follows: the cells were almost completely covering the plate and had a few noticeable cilia present. This is what we expected because they are non-cancerous cells and were getting too crowded, so upon the culture reaching confluency, the cells began to exit the cell cycle, entering G0 (Figure 7). For the second column, which was grown in the 10% growth serum with Dexamethasone, results were as follows: the cells were completely covering the plate and had an abundance of cilia present. In comparison to the 10% without Dexamethasone, there are more cilia, which we can deduce may be due to the addition of Dexamethasone (Figure 8). For the third column, which was grown in the 1% growth serum without Dexamethasone, results were as follows: there were many cells covering the plate, with a slight reduction compared to the 10% conditions (Figure 9). Additionally, there were as many cilia as there were on the 10% with Dexamethasone. This is one of the most important parts of the experiment because there is no way to place tumor cells in the body in a starvation environment, so to see the 10% with Dexamethasone and the 1% without starvation have

similar amounts of cilia is notable. For the fourth column, which was grown in the 1% growth serum with Dexamethasone, results were as follows: the cells looked very clumpy. There were no cilia present and overall, the cells did not look healthy (Figure 10). This could be due to the cells not being able to metabolize the Dexamethasone without sufficient nutrients.

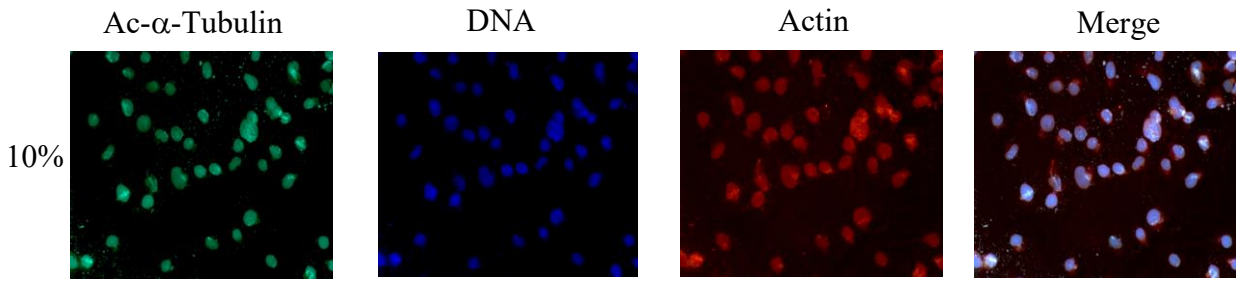

Figure 4. PC3 cells grown in 10% serum for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and Rhodamine was used to stain actin (red). The fourth image represents the merge of all three previously mentioned stains. These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

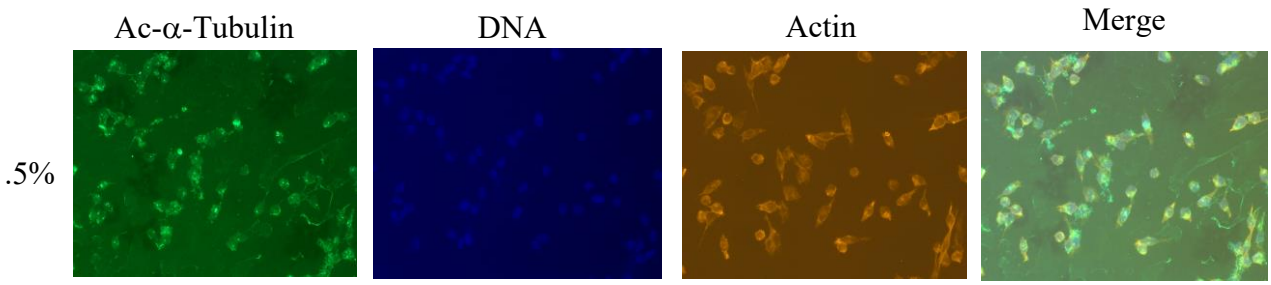

Figure 5. PC3 cells grown in 0.5% serum for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and Rhodamine was used to stain actin (red). The fourth image represents the merge of all three previously mentioned stains. These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

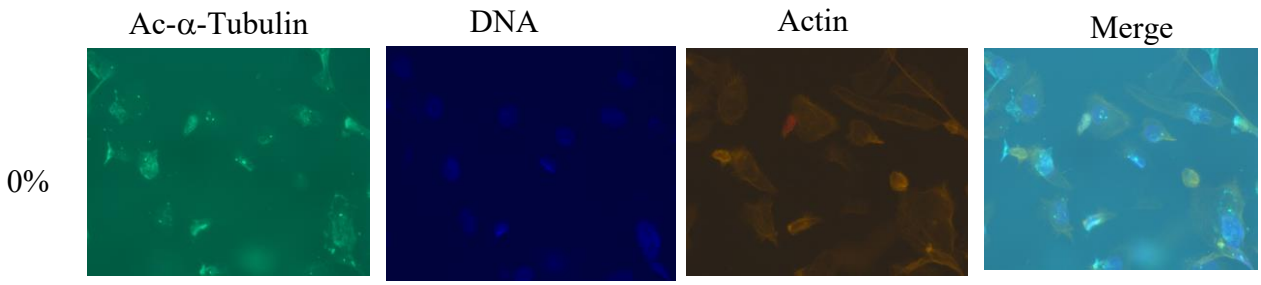

Figure 6. PC3 cells grown in 0% serum for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and Rhodamine was used to stain actin (red). The fourth image represents the merge of all three previously mentioned stains. These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

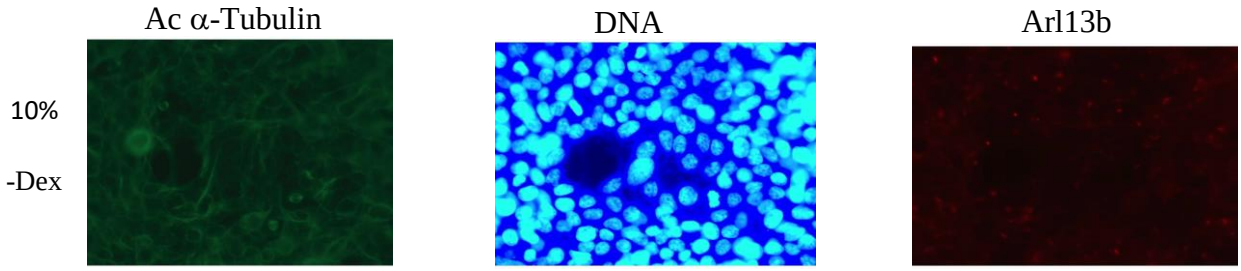

Figure 7. HEK 293 cells grown in 10% serum without Dexamethasone for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and anti- Arl13b antibody was used to stain the cilia (red). These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

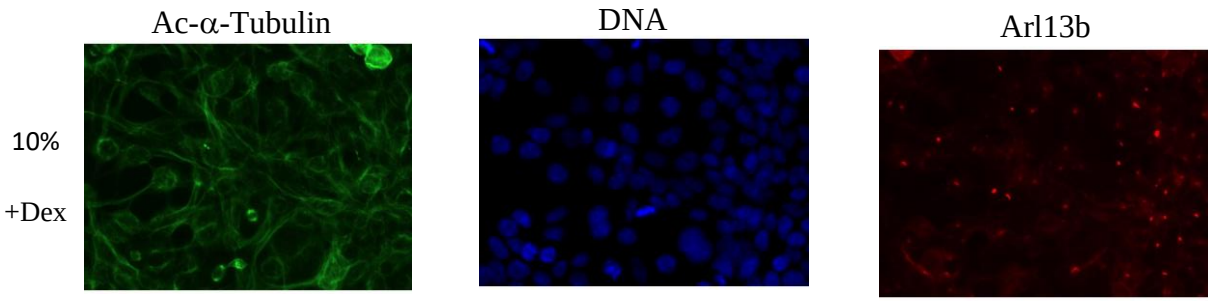

Figure 8. HEK 293 cells grown in 10% serum with Dexamethasone for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and anti- Arl13b antibody was used to stain the cilia (red). These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

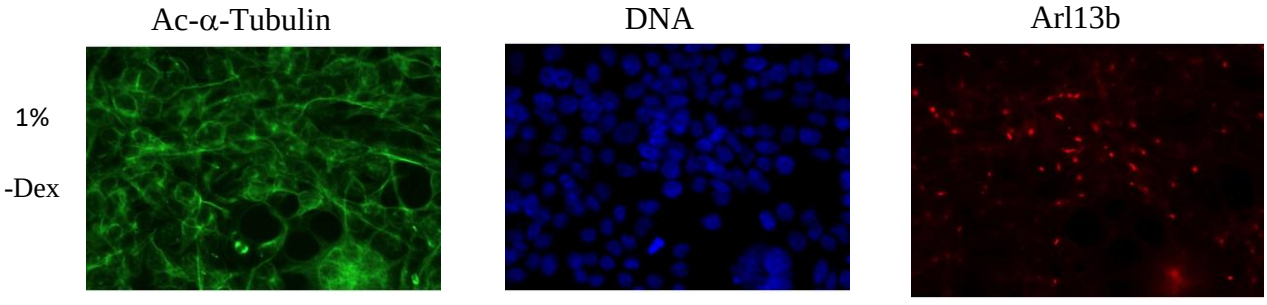

Figure 9. HEK 293 cells grown in 1% serum without Dexamethasone for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and anti- Arl13b antibody was used to stain the cilia (red). These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

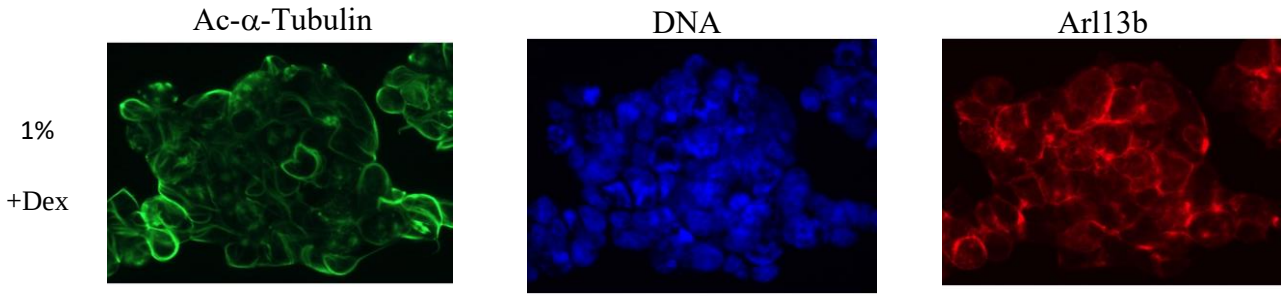

Figure 10. HEK 293 cells grown in 1% serum with Dexamethasone for 48-hour. An anti-acetylated α-tubulin antibody was used to visualize ciliary tubulin (green), DAPI was used to stain DNA (blue) and anti- Arl13b antibody was used to stain the cilia (red). These images were captured with a Zeiss AXIO imager A2 microscope and the ZEN software.

4. Discussion and Conclusion

The primary objective for this experiment was to determine whether prostate cancer cells can be induced to undergo ciliogenesis, either through starvation and/or Dexamethasone treatment. Cancer cells negate their surrounding environment and in turn grow on top of each other rapidly, as long as they have nutrients they will continue to divide. The hypothesis was: if prostate cancer cells had primary cilia then they would be in G0 and therefore stop dividing. Overall, the major findings are that by inducing primary cilia we were able to stimulate moderate ciliogenesis via starvation and pharmaceutical drugs, and thus halt cell division. This study further confirmed that PC3 cells are able to produce and HEK 293 cells are able to produce cilia with starvation and Dexamethasone. The next step is to possibly only target the PC3 cells and add Dexamethasone. Additionally, the use of a confocal microscope will result in higher resolution images of the primary cilia

Dexamethasone has been used as a chemotherapy for other cancers, this is exemplified in other research studies. When looking into non-small cell lung cancer (NSCLC) reports showed that dexamethasone could possibly be used as an anti-estrogenic agent. At low micromolar levels Dexamethasone was also able to seize cell proliferation.[10] Moreover, studies suggest that Dexamethasone affected cell cycle progression in human airway fibroblasts isolated from patients suffering with asthma.[11] This is important because it showcases that other conditions also use Dexamethasone to examines its effect on the cell cycle. Lastly, Dexamethasone was used as a possibly chemotherapy in acute myeloid leukemia. In the end the study concluded that Dexamethasone "can reduce the frequency of leukemic […]. Dexamethasone also demonstrated antileukemic activity in *NPM1*-mutated samples. Dexamethasone may improve the outcome of acute myeloid leukemia patients receiving intensive chemotherapy".[12]

If PC3 cells are proven to grow primary cilia successfully with the addition of Dexamethasone we will then try using an African American cell line with the same experiment. Similar to brachytherapy, it may one day become possible to use Dexamethasone as a chemotherapeutic agent. In conclusion, this experiment with PC3 and HEK 293 cells, has contributed to our knowledge of PC3 cells and prostate cancer research as it relates to primary cilia. We hope to contribute more knowledge to the field of the cell biology of prostate cancer that will ultimately lead to helping individuals experiencing the devastating effects of prostate cancer: the elderly, the financially disadvantaged, and African American men.

5. References

1. American Cancer Society, "Prostate Cancer Risk Factors", March, 2016, www.cancer.org/cancer/prostate-cancer/causes-risks-prevention/risk-factors.html
2. Ibid.
3. Ibid.
4. Ibid.
5. Ibid.
6. Hassounah N.B. and others, "Primary Cilia Are Lost in Preinvasive and Invasive Prostate Cancer," PLoS ONE, 8, no. 7 (2013): 1.

7. Wang L. and Dynlacht B.D., “The regulation of cilium assembly and disassembly in development and disease,” Development, 145, no. 18 (2018): 9.

8. Mitchison H. M. and Valente E. M., “Motile and non-motile cilia in human pathology: from function to phenotypes,” Journal of Pathology 241 (2017): 296.

9. Khan N. A. and others, “Identification of drugs that restore primary cilium expression in cancer cells,” Oncotarget, 7, no. 9 (2016): 9975.

10. Wang, Li-Jie and others, “Dexamethasone Suppresses the Growth of Human Non-Small Cell Lung Cancer via Inducing Estrogen Sulfotransferase and Inactivating Estrogen,” Acta Pharmacologica Sinica, 37, no. 6, (20160: 846–856.

11. Fouty, and others, “Dexamethasone Can Stimulate G1-S Phase Transition in Human Airway Fibroblasts in Asthma,” European Respiratory Journal, 27, no. 6, (2006): 1160.

12. Bertoli, Sarah and others, “Dexamethasone in Hyperleukocytic Acute Myeloid Leukemia.” Haematologica, 103, no. 6 (2018), 990.