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Keeping an Open Mind: Challenges and Mysteries in Cancer Cell Biology Research

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Commentary

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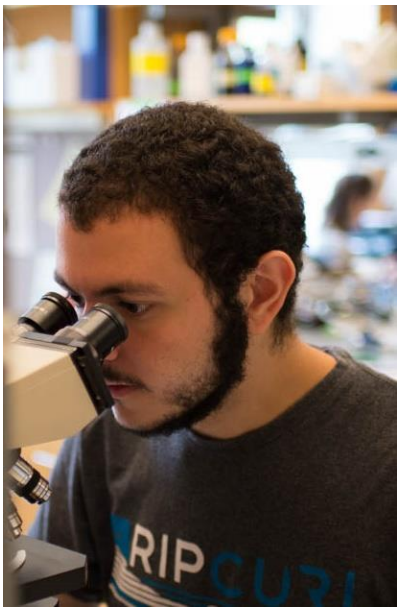
—Yusuf Ebrahim

Cancer arises after a series of mutations or other alterations allows cells to bypass their normal growth checkpoints and divide freely in the body. The body aims to prevent tumors from forming by protecting the integrity of its cells' DNA. One protein, p53, is so vital in this role that it is often referred to as the "guardian of the genome." In fact, more than half of all human cancers are associated with malfunctions that disrupt p53 function (1).



The author in the lab.

In a normal cell, p53 is activated during the growth cycle and, if the cell has substantial mutations in its DNA, p53 journeys from the cell's cytoplasm to its nucleus to trigger apoptosis (programmed cell death). Since normal p53 prevents tumors by halting their spread, abnormalities in p53 might permit a cell to become cancerous. In some of the cancerous cells I studied with Dr. Walker, the seemingly unrelated protein mortalin, which binds to p53, is overexpressed. This keeps p53 from entering the nucleus, and allows cancerous cells to survive and divide (2). My research goal has been to investigate this interaction, known as the "p53-mortalin complex," in patient-derived and other samples of human cancer cells.

I received a Summer Undergraduate Research Fellowship (SURF) in 2016 to work in Professor Chuck Walker's cell biology lab, where I had been involved since 2014. I worked under the guidance of Dr. Walker and alongside other colleagues in the lab. My work during that time focused on culturing cancer cells and investigating p53 within them. The various surprises I experienced while working on my SURF project taught me that research won't always be as clear-cut as one might expect. I learned the importance of keeping an open mind and considering the possibility of obstacles and unexpected outcomes in order to make sense of conflicting results.

Project and Challenges

Two chemical agents used in our lab, MKT-077 and withanone, have the potential to disrupt the p53-mortalin complex by binding to mortalin. This means that they might be able to free p53 and allow it to enter the nucleus to kill the cancer cells (2, 3). Because MKT-077 and withanone target the very mechanism that allowed the cancer to form in the first place, they should have the ability to discriminate between cancerous cells and healthy cells. This is in contrast to many chemotherapies in use today, which target any actively dividing cells and often cause damage to healthy parts of the body, promoting side effects such as hair loss or increased risk of infection. Withanone is an extract of the plant ashwagandha, which has been used in traditional Indian herbal medicine for many years. Although it has not been tested extensively with regard to the p53-mortalin complex, evidence suggests that withanone should be effective in cases where the complex is present (3).

My project had two objectives. First, I sought to confirm the presence of the p53-mortalin complex in the cells I was planning to use. Second, I tried to disrupt the complex using MKT-077 and withanone and determine the effectiveness of these agents in allowing p53 to move to the nucleus and trigger apoptosis. I planned to designate groups of cells as untreated, MKT-treated, or withanone-treated. For each group, I chose a series of analytical techniques that could pinpoint p53 in the cell (to see whether it was stuck in the cytoplasm or already in the nucleus) and determine the levels of cell death by apoptosis.

Initially I planned to perform this experiment on samples of cancer cells derived directly from acute myeloid leukemia (AML) patients. Results derived from patient samples can be more valuable than those derived from established cell cultures available from the American Type Culture Collection (ATCC), a library of cell lines. AML cells from patient samples are more like the cells that would be treated in a real cancer patient, because they haven't been growing in flasks for too long. They also come from a variety of patients rather than only one, so they provide more widely applicable results regarding the effects of the chemical agents. However, these patient samples are limited in number. We realized our supply was low, so we would have little room for error in our experiments. Dr. Walker and I decided at the start of the summer to first run the experiment with a neuroblastoma cell line (called IMR-32), in order to make sure that our techniques and materials worked properly before moving to the patient samples.

Cell lines, like IMR-32, are also derived from cancer patients, but they are grown in flasks for long periods, which could cause them to become different over time. IMR-32 cells were derived from a child who had neuroblastoma in 1967. While cells from culture may have changed from their original source, they can be grown indefinitely, so if we needed to repeat part of the experiment, we could simply prepare a new flask. In addition, because all IMR-32 cells were derived from the same patient many years ago, they are roughly uniform and the results should show that the p53-mortalin complex exists in either all of them or none of them, making our results easier to interpret. By changing the sample cells, I learned that I had to be flexible and willing to deviate from the original design of the experiment. The proposal I wrote for my project was theoretical and didn't consider the specific

resources of the lab or the needs of the overall research project; when that information was brought to light, we had to adapt and make some tweaks to the experiment.

At the beginning of the summer, I had trouble starting up a cell line from our lab's vials of frozen cells. I tried a few times to thaw these IMR cells, but initially they did not survive. Sometimes the cells would give us false hope by surviving for a day or two before failing. Under the microscope, the cells would start off looking healthy—round and firmly adhered to the bottom of the flask. But within a few days, most of the cells would be floating and fragmented.

I was using vials that we'd frozen down ourselves in the past. We ordered a fresh vial of IMR cells from the ATCC, a "library" that stores cells that are close to the original source of the line. This fresh vial was successful, and I kept a culture of IMR cells going through the summer. We think that our original vials weren't working due to an incident that left the liquid nitrogen tank (where the cells were stored) nearly empty the preceding fall, and due to a possible error made when the cells were being frozen down. This experience led me through a sequence of troubleshooting: I initially questioned the technique I was using to thaw the cells, then the culture medium we were feeding to the cells, and finally the frozen vials themselves. After seeing that what I was doing wasn't working, I realized that I had to make a change each time rather than repeat the same procedure.

Starting the Treatments

When I had the cells growing successfully, I started treating them with withanone to observe its effects, and I met my next obstacle. After the first treatment, I noticed under the microscope that the cancer cells looked as happy as ever; subsequent tests showed no significant difference in the levels of apoptosis, or cell death, between the withanone-treated cells and the untreated cells. Since the treatment seemed ineffective, my first thought was to question the vial of withanone. After inspecting it more closely, I discovered that it had expired. We ordered a new vial of withanone and repeated the treatments, but even after increasing the treatment concentration and duration to the maximum we had planned, the withanone-treated cells and the untreated cells appeared the same. The withanone was just not working. This result confused me; prior knowledge told us that withanone was supposed to disrupt the p53-mortalin complex in cells that have it (3). So if the withanone was not working, and we were performing the experiment correctly, it might mean that, contrary to our previous suspicions, the IMR cells don't really have the p53-mortalin complex.

Regardless, I didn't think more of the matter and carried on. Next, I used a technique called immunocytochemistry to stain p53 in the IMR cells, and I used a microscope to observe the position of p53. Even in the untreated cells, the centers of the cells lit up under the microscope after the stain, which would indicate that most of the p53 was located in the nuclei of the IMR cells rather than in their cytoplasm (Figure 1). At the same time, other members of the lab had run a number of immunoblots, which can detect the presence of p53 in a solution of proteins, on separate solutions containing the cytoplasm and nuclei of the IMR cells. These blots showed that there was a lot of p53 in the nuclei of the IMR cells and a relatively small amount of p53 in their cytoplasm. Since mortalin is

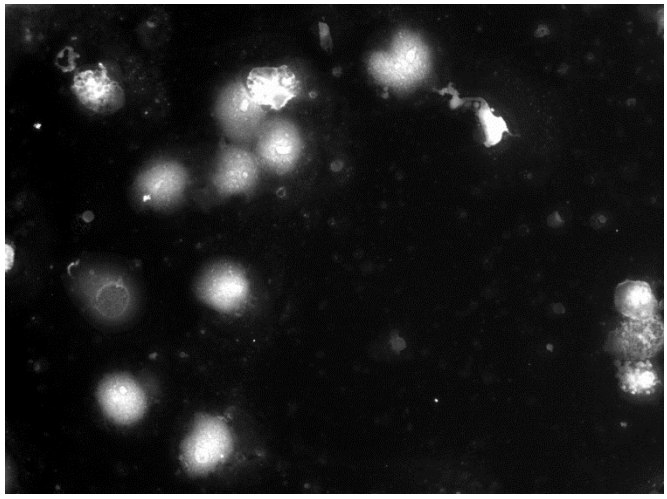


Figure 1. IMR-32 cells stained for p53, viewed under fluorescent light. The glowing regions represent the position of p53 in the cells. The centers of the cells are lighting up more than their outer edges; if the stain was performed correctly, this can be used to demonstrate that p53 is located in the nucleus of IMR-32 cells.

generally only found in the cytoplasm, this would further imply that the IMR cells didn't have the p53-mortalin complex.

The evidence suggesting that the IMR cells might not have the p53-mortalin complex was throwing me off, because, despite seeming to be self-consistent, it contradicted not only some literature we'd read, but also my own past findings from the IMR cells (4). However, I think that my past results were not as comprehensive as those during my summer work. Since I was facing some conflicting evidence about whether or not the p53-mortalin complex was in the IMR cells, I needed to do some further testing to find out what was true.

Next Steps

Because our research focuses on the p53-mortalin complex, the finding that this complex might not be present in the IMR cells—if true—would mean that the IMR cells won't be relevant to our next research steps. However, I believe that even a negative result—the lack of something—is important, because we could not have known it otherwise. Due to the earlier setbacks with starting the cells up and repeating the withanone treatments, we didn't have a chance to test the MKT on the IMR cells during the summer. Nevertheless, we gained a lot more experience with and knowledge about the experiments and analyses we were running.

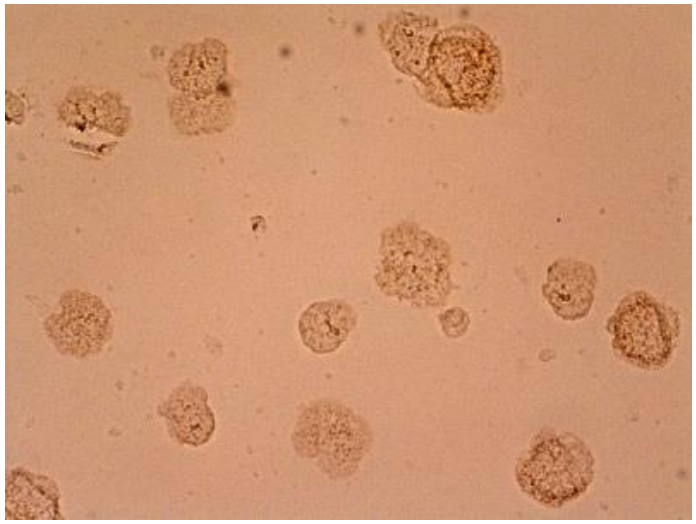


Figure 2. IMR-32 cells stained for p53, viewed under normal light. The brown spots represent the position of p53 in the cells. The brown regions form a ring around the outer edges of most of the cells; this would contradict previous findings and show that p53 is located in the cytoplasm of IMR-32 cells.

Our next step will be to run similar tests on the AML patient samples. I'm excited to investigate the patient samples, because they are so different to work with, but I am also interested at looking further at what's going on in the IMR cells. In December 2016, I repeated the immunocytochemistry for the IMR cells with a modified staining procedure; this showed p53 in the cytoplasm of the IMR cells (Figure 2). I used a more definitive staining procedure again in February 2017, which also showed p53 in

the cytoplasm of the IMR cells (Figure 3). My next step, therefore, will be to investigate whether it is mortalin that is responsible for keeping p53 in the cytoplasm, by using a technique called co-immunoprecipitation, which can identify whether proteins are bound together. After that, depending on the results, I might look more closely at the effects of MKT-077 and withanone on the IMR cells.

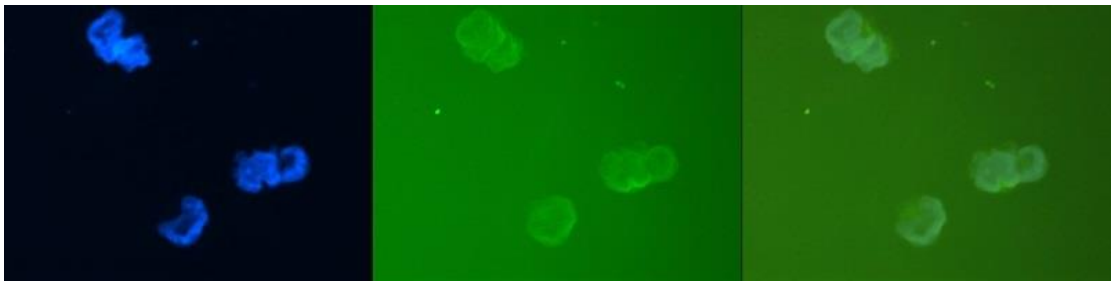


Figure 3. IMR-32 cells stained for p53, viewed under fluorescent light with two different filters. The blue regions in the image on the left represent the cells' nuclei, whereas the green regions in the center image represent the position of p53 in the cells. The image on the right is the result of merging the left and center images. This shows that p53 is located in the cytoplasm of IMR-32 cells, because most of the green regions (p53) are concentrated outside the blue regions (nucleus).

Final Thoughts and Lessons

I learned a number of valuable lessons by performing this research, in addition to new lab methods. I worked mostly independently on my project, with guidance from Dr. Walker, which allowed me to make choices such as restructuring parts of the experiment based on the results I was seeing. Most of all, I learned that it is important to actively keep an open mind, especially in science. It took me a while to understand that withanone was not effective against the IMR cells, despite changing the experiment repeatedly—new withanone, longer incubation times, higher concentrations—with the assumption that I was running some part incorrectly because I wasn't seeing the result I expected. It was good that I repeated and changed the experiment to rule out other possibilities, but when I accepted that the withanone just wasn't working, I started thinking about why that might be and changed my previous ideas about the IMR cells. In other words, I never expected to get unexpected results. This lesson will be important in my goals moving forward—I hope to become a doctor, and the ability to question myself and my previous ideas will be equally valuable in that setting. Because of the support of my lab mates and my mentor, my undergraduate research in Dr. Walker's lab has been my favorite experience at the University of New Hampshire. I eagerly look forward to continuing my work in the lab.

I would like to thank Dr. Walker for always instilling curiosity in me, guiding me in my research, and providing me with invaluable support in my journey. I would also like to thank the Hamel Center for Undergraduate Research for giving me the opportunity to conduct the research and making it all possible. Finally, I would like to thank all the students, former students, and staff I've worked with in the lab, who were always helping me in my project and providing me with company: Bria Frehner, Jasmina Cesko, Brianna Looney, Seth McNutt, Andrew Morin, Harrison LeFlem, Professor Anne Böttger, and Dylan Laprise.

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Author and Mentor Bios

Yusuf Ebrahim, from Portsmouth, New Hampshire is a member of the University Honors Program majoring in biomedical science and minoring in chemistry and psychology. Yusuf will graduate in May 2018 and hopes to become a doctor. According to Yusuf, his Summer Undergraduate Research Fellowship (SURF) project allowed him to conduct meaningful research that ties to what he hopes to do as a career. Yusuf has always been interested in science and is particularly intrigued by cell biology. He found the opportunity to study cancer cells to be not only a medically relevant experience but also one that allowed him to practice skills he will need in the future. Yusuf says that he found it incredibly satisfying to run an experiment multiple times and then finally get a result from the trials, especially when the result was different from the expected findings. By submitting to *Inquiry,* Yusuf hoped to share with others the importance of keeping an open mind while researching and to demystify the research process for readers.

Professor emeritus **Charles Walker** has taught molecular, cellular, and biomedical sciences at the University of New Hampshire (UNH) for forty-one years. He teaches honors introductory molecular and cellular biology as well as eukaryotic cell and developmental biology. Professor Walker has mentored approximately 300 undergraduate researchers in his time at UNH. For Professor Walker, “It is always a superb pleasure and an honor for me to work with students as they develop from their freshman year through to their employment as physicians, faculty at other universities, employees at various biotech and pharmaceutical companies, or as educators in the biological sciences.” He first became involved with Yusuf’s research after meeting him in honors biology, and he is helping Yusuf on the path to success that so many of his other students have followed.