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FIRST PERSON

First person – Stephanie Bouley

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping researchers promote themselves alongside their papers. Stephanie Bouley is first author on 'Chemical genetic screens reveal defective lysosomal trafficking as synthetic lethal with NF1 loss', published in JCS. Stephanie conducted the research described in this article while a PhD candidate in Yolanda Sanchez's lab at Geisel School of Medicine at Dartmouth College, Hanover, USA. She is now a post-doctoral research fellow in the lab of James A. Walker at Massachusetts General Hospital Center for Genomic Medicine, Boston, MA USA, where she is interested in identifying novel therapeutic targets for treating neurofibromatosis type 1-associated tumors.

Stephanie Bouley

How would you explain the main findings of your paper in lay terms?

Our research focused on finding new therapeutic targets to selectively treat tumors lacking the protein neurofibromin (NF1), which regulates the Ras cell signaling pathway. Ras activity is responsible for many cellular processes, and uncontrolled Ras activity leads to uncontrolled cell growth and tumor formation. Loss of NF1 is the defining hallmark of the genetic condition neurofibromatosis (NF) type 1, for which there are limited treatment options. We identified a chemical compound called Y102 that could selectively kill cells lacking the *NF1* gene without affecting normal cell growth. We found that treatment with Y102 caused an increase in markers of stress associated with dysfunction of a process known as autophagy, which cells use to degrade and recycle cellular components. We also observed significant damage to mitochondria and alterations in mitochondrial localization within the cell, suggesting that Y102 impacts a selective form of autophagy that targets the mitochondria, known as mitophagy. We then performed two separate analyses to identify the molecular target of Y102, and through these experiments we identified this target as BORC, which is a complex involved in late-stage autophagy and mitophagy. This work benefits not only the neurofibromatosis type 1 research community by identifying a novel therapeutic strategy in treating *NF1*-deficient tumors, but also the broader cancer research community, as loss of *NF1* occurs in several cancer types. Finally, the compound Y102 could be developed into a drug using this data, allowing for a unique new strategy to inhibit autophagy and mitochondrial clearance.

Were there any specific challenges associated with this project? If so, how did you overcome them?

One of the greatest challenges associated with this project was also the spark that attracted me to this work in the first place. Frequently in research, a drug is chosen for development because we already know what it does, and we want to determine if it has an effect in a certain cell type or under specific conditions. In this instance, we had an undeveloped small molecule that we knew could selectively kill *NF1*-deficient tumor cells, but we had no idea how. We had to investigate a number of potential mechanisms of cell death before we realized the connection to autophagy. Implementing several different proteomics strategies to identify potential targets of Y102 is what allowed us to propel this project forward and ultimately conclude that BORC was the drug's target.

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

My favorite experiment that I conducted for this project was a complicated immunofluorescence staining utilizing a tagged version of both a subunit of BORC and the compound Y102 to demonstrate colocalization. I have always loved confocal microscopy as a means of visualizing what exactly is taking place in a cell. The staining and imaging procedures themselves were relatively easy to carry out but determining the best location on the molecular structure for tagging Y102 involved designing, synthesizing and testing several analogs, which granted us a lot of insight into its chemistry. This experiment taught me the importance of perseverance and determination in research, which is something that I continue to hold on to in my current scientific pursuits. Visualizing the colocalization between Y102 and BORC for the first time was extremely validating and rewarding and it still makes me smile to this day.

Why did you choose Journal of Cell Science for your paper?

Journal of Cell Science has a tremendous history with respect to the importance of microscopy in understanding cell biology, having originally been established as The Quarterly Journal of Microscopical

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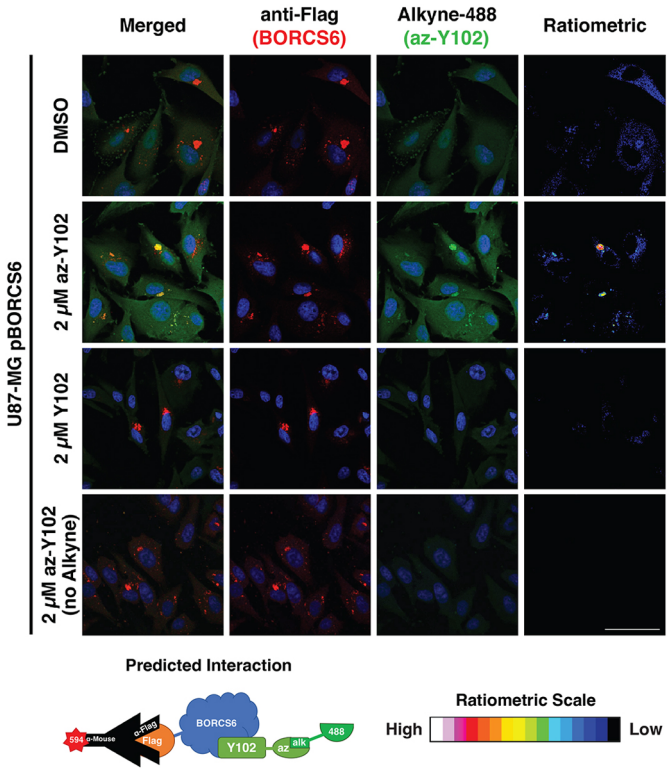

U87-MG cells expressing FLAG-tagged BORCS6 were treated with azide-tagged Y102, unmodified Y102 or DMSO. Afterwards, azide-tagged Y102 (az-Y102) was labeled with alkyne-488 via click chemistry (green), and BORCS6 was visualized using anti-FLAG (red). Nuclei are labeled in blue. Ratiometric images comparing the colocalization between az-Y102 and BORCS6 were generated using Fiji software. Resulting fluorescence is displayed as intensities (16-color; bar below). Scale bar: 50 µm.

Science in 1853. Since then, Journal of Cell Science has published high-quality, peer-reviewed papers focused on all aspects surrounding the structure and function of the cell. The work carried out in this project relied heavily on microscopy to understand the effects of Y102 treatment on the expression of different proteins and organelle localization in *NF1*-deficient cells, which is why I think Journal of Cell Science was a great fit for this publication.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

I am fortunate to have been mentored by many successful researchers throughout my research career. As a first-generation college student, my undergraduate mentors played a significant role in helping me develop my scientific prowess and propelling me into a research career. My graduate advisor, Dr Yolanda Sanchez, was one of the first female scientists I met who was thriving in academia, demonstrating what hard work, dedication and resolve can help you achieve. Yoli often reminded us that if you are striving towards progress, you are still succeeding – even if you don't feel like you are reaching your research goals. My current mentor, Dr James Walker, is one of the first PIs I have met who makes the job look easy, because he loves the research. Jim is fully devoted to the projects being conducted in his laboratory but perhaps even more importantly, he is equally committed to his team of investigators. Outside of research, he gives career and life advice, celebrates our personal and professional achievements and shows genuine care for our well being, which makes his lab a great place to be.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I grew up with several family members who were ill with genetic conditions or diseases with few therapeutic options available to them. It was difficult to watch them struggle and suffer because science had not yet found a solution to provide them with better quality of life, treatments and cures. I know the importance of drug discovery and translational research because of their first-hand experiences. This is what drew me into the neurofibromatosis research field, as there is no cure for NF and very limited treatment options. I have found that the NF community is one of the most welcoming research communities to be a part of, because everyone involved is committed to the patients and their families. I'm grateful to be a small part of this group working to end NF.

Who are your role models in science? Why?

I have always had a deep love for Gregor Mendel, the Augustinian friar known as the father of modern genetics. It was while he was serving as a secondary school teacher that he began his seminal studies on the transmission of hereditary traits in plant hybrids. While we recognize the importance of his work today, at the time, the results of his experiments were significantly underappreciated even though his paper had been distributed to 120 institutions and he personally sent 40 copies to the most renown botanists of the age. It took three and a half decades after its publication and a decade after his death for Mendel's work to begin to get the recognition it should have initially received. Gregor Mendel is one of my favorite scientists because of how he approached his work and life in general. He is quoted to have said the following: "Even though I have experienced some dark hours during my lifetime, I am grateful that the beautiful hours have outweighed the dark ones by far. My scientific work has brought me great joy and satisfaction; and I am convinced that it won't take long that the entire world will appreciate the results and meaning of my work… my time will come." I think this is a great outlook to have in science, as not everything will work out, but when it does, it should bring you joy and a sense of accomplishment.

What's next for you?

My immediate plans are to continue my research on NF1 as a post-doc at Massachusetts General Hospital. My long-term goals are to maintain a research lab while teaching at an undergraduate institution, as I want to educate and mentor the next generation of scientists and physicians. By developing an academic environment at the undergraduate level where students can be educated on the intricacies of molecular biology and genetics, while fostering knowledge on proper research strategies and ethical considerations in the laboratory, I aim to encourage novice researchers to partake in novel rare disease research at an early career stage.

Tell us something interesting about yourself that wouldn't be on your CV

I started teaching people how to play Dungeons & Dragons during the COVID-19 pandemic to help foster community in the absence of physical presence. I play in and lead a few games, and I've also taken up resin dice making. My Westie Daisy Grace is the logo on my dice and she's wearing a doctoral tam as a nod to my degree.

Reference

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