Over the past 3 months I have been honored to study and work in a contemporary laboratory at Harvard Medical School thanks to the generosity of the Summer Research Opportunity Program in Pathology (SROPP) sponsored by the American Society for Investigative Pathology (ASIP). I was mentored and supervised daily by Dr. Diane Bielenberg, Ph.D., a cancer biologist in the Vascular Biology Program at Boston Children’s Hospital. My summer experience was enriched by participating in the Continuing Umbrella of Research Experiences (CURE) Program at the Dana Farber Harvard Cancer Center where I was immersed in a diverse community of nearly forty summer students. Weekly activities included Monday seminars focused on cancer research and cancer treatment given by local experts, a journal club where we discussed cancer-related published articles from basic research to clinical trials, evening seminars that included a panel discussion on career opportunities in biomedical sciences, weekly lab meetings in which we took turns sharing our data, and a book club where we read and discussed Pandora’s DNA: Tracing the Breast Cancer Genes Through History, Science, and One Family Tree by Lizzie Stark.

However, the majority of my time was spent in the laboratory. My project focused on the role of the cell surface receptors called Neuropilins in renal function. Two Neuropilin receptors called NRP1 and NRP2 have disparate roles in angiogenesis and cancer progression depending on which ligand they bind. The VEGF family of ligands bind to NRPs to promote angiogenesis, the sprouting of new blood vessels, and thereby tumor growth. Whereas, the Semaphorin 3 (SEMA3) family of ligands bind to NRPs to inhibit neovascularization and therefore are potent anti-cancer drugs in preclinical trials. A recent Phase Ib clinical trial using anti-NRP1 antibodies in combination with anti-VEGF antibodies and chemotherapy (Cancer Chemother Pharmacol 73(5):951-60, 2014) was discontinued due to the high level of proteinuria seen in human cancer patients. Based on this human trial, we hypothesized that NRP1 may be highly expressed in podocytes, specialized epithelial cells in the glomeruli of the kidney that are involved in protein filtration from the blood into the urine. Normally, high molecular weight proteins are not found in the urine, but if a drug causes renal toxicity then the podocyte-endothelial barrier may become damaged and release proteins into the urine, a condition called proteinuria.

Using immunoblotting I examined the expression of Nrp1 and Nrp2 in various mouse organs. I determined that Nrp1 was highly expressed in kidney protein lysates, whereas Nrp2 was only weakly expressed. To localize the expression of the Nrp receptors within the kidney, I used immunohistochemistry in paraffin and frozen tissue sections. Nrp1 was highly expressed in podocytes in the glomeruli and also found in epithelial cells of the renal tubules and in endothelial cells of blood vessels. Nrp2, on the other hand, was only found in endothelial cells, in particular in veins and lymphatic vessels in the kidney. Our immunohistochemistry results were confirmed by staining kidneys from transgenic Nrp2<sup>Ires</sup> mice with X-gal reagent to locate cells with a beta-galactosidase gene insert (denoting Nrp2 expression). To further examine the function of the Nrp receptors in vivo in the kidney, I performed protein assays to quantify the amount of protein in the urine collected from mice housed in metabolic cages. Mouse urine is much more concentrated than human urine. When I compared urine from wild-type mice (Nrp2<sup>+/+</sup>) and mice lacking Nrp2 expression (Nrp2<sup>−/−</sup>), I found low...
levels of protein in both conditions suggesting that targeting the NRP2 receptor, unlike the NRP1 receptor, may not cause renal toxicity. However, when we injected mice systemically with adenoviral constructs encoding SEMA3 proteins, we did see an increase in urine protein levels indicating that these inhibitory proteins may cause podocyte damage or interfere with filtration function. Taken together, our data suggest that anti-cancer therapies should target the NRP2 receptor rather than the NRP1 receptor to limit toxicity while maintaining efficacy.

Personally, this summer internship provided by ASIP gave me the unique opportunity to gain hands-on laboratory experience in a fast-paced and energetic environment. I learned numerous laboratory techniques and interacted daily with students, post-doctoral fellows, clinical fellows, and professors at every level. Most importantly this experience taught me to think carefully, to analyze my data critically, and to prepare my results in a timely manner. At the end of the summer, I prepared an abstract and presented my research in the form of an E-poster (shown above in the photo) to the Harvard Community. I plan to present my studies at the upcoming ABRCMS meeting in 2016 and at Experimental Biology in 2107. My future goals are to continue a career in the biomedical sciences and to apply to an MD/PhD program following my graduation from Tufts University in 2019.