The Bee Foundation Brain Aneurysm Research Grant 2019/20

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Molecular basis of endothelial dysfunction for personalized risk stratification in intracranial aneurysms

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Project Overview

When an intracranial aneurysm ruptures, it causes a hemorrhagic stroke resulting in significant morbidity and mortality. Approximately 2-3% of the population develops an intracranial aneurysm, and 30,000 aneurysms rupture annually in the United States. Early detection and treatment of aneurysms can prevent hemorrhagic stroke. However, treatment requires an invasive surgical procedure, which is potentially risky and costly. The management of incidentally-discovered small aneurysms remains controversial. In most patients with small aneurysms, no treatment is recommended; for large aneurysms, or aneurysms in patients with additional risk factors, prompt intervention is indicated to prevent rupture. A validated scoring system called the PHASES aneurysm risk score has been developed to predict risk of future aneurysm rupture. Nevertheless, this and other aneurysm size-based scoring systems are imperfect since the majority of ruptured aneurysms are small and currently there is no robust method of predicting an individual aneurysm's likelihood of rupture. Developments in understanding the genomics of aneurysm growth and rupture coupled with the development of a minimally invasive endovascular method of obtaining endothelial cells from aneurysms and other healthy blood vessels have opened new avenues for research.

Endothelial cell genetics have been proposed to play a critical role in aneurysm formation, growth and rupture. Aneurysms occur at sites of increased hemodynamic stress and we hypothesize that specific endothelial markers of vascular inflammation, dysfunction and differential response to hemodynamic stress are dysregulated during aneurysm pathogenesis. To access these cells for analyses, we have optimized a recently developed minimally-invasive endovascular endothelial cell collection technique to obtain aneurysmal endothelial cells during cerebral angiography. This is important in an era when most aneurysms are treated

with endovascular techniques and research has relied on surgical specimens. This novel technique can be used to obtain cells from both aneurysm and healthy tissue samples.

The goal of this research is to build genomic transcriptome profiles of key vascular factors from both aneurysmal and non-pathological tissue. These expression profiles will be compared between low- or high-risk aneurysms (scored using PHASES) in both ruptured and unruptured states. Preliminary results suggest that distinctive transcriptome profiles for individual aneurysms will be obtained, which can be used to identify aneurysms at low- and- high risk of rupture, thus facilitating treatment decisions that are personalized for the individual patient and aneurysm of interest.

Aim 1: Collect endothelial cells during endovascular treatment of low-risk and high-risk aneurysms (as defined by PHASES score).
Aim 2: Perform targeted transcriptome measurement of collected endothelial cells by single-cell RNA sequencing (RNA-Seq).
Aim 3: Compare the differential profile of key vascular factors of aneurysmal endothelial cells in unruptured, ruptured, low- and high-risk aneurysms.

Current Progress

Aim 1: Collect endothelial cells during endovascular treatment of low-risk and high-risk aneurysms (as defined by PHASES score).

The first step in our work involved establishing a safe, reliable and reproducible method to collect endothelial cells which are adherent to aneurysm coils during endovascular aneurysm treatment (Fig 1). Through a process of optimization, we determined that only the first coil which has had significant contact with the aneurysm wall reliably yielded endothelial cells. Control endothelial cells were also obtained from the access sheath. Once collected, cells were stripped from the coils and immune-labelled in preparation for fluorescent-activated cell sorting (FACS). With FACS, cells were separated based on their molecular identify and single endothelial cells were deposited into a PCR tube (Fig 2). To date, we have collected coils and cells from 15 patients with both unruptured and ruptured aneurysms without any adverse clinical events.

Fig 1. Dr Michael Levitt and Dr Ryan Kellogg preparing to coil a posterior communicating artery aneurysm, from which a non-deployed coil was obtained and processed for endothelial cell collection.

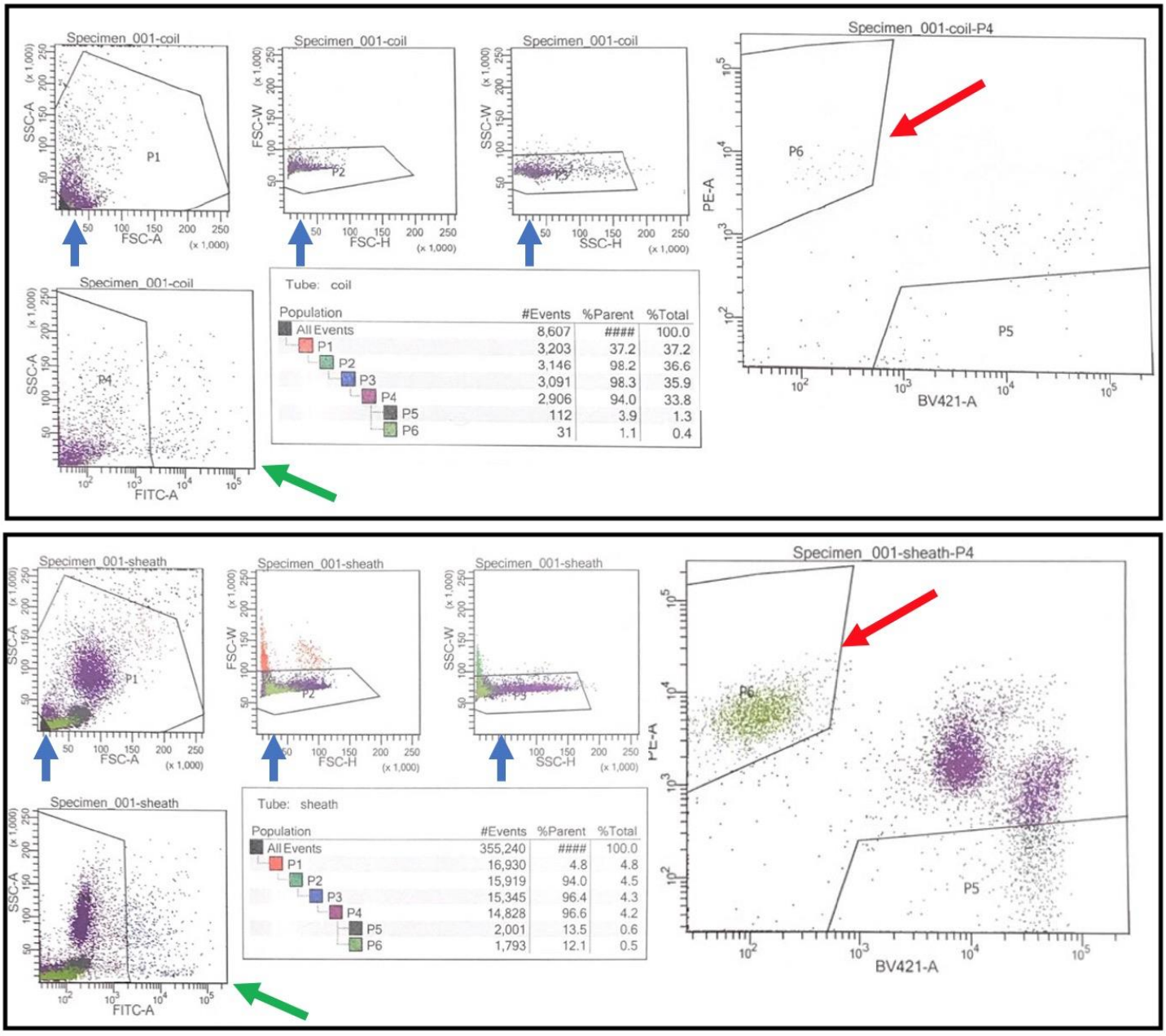

Fig 2. Fluorescence Activated Cell Sorting (FACS)
Once the cells pass the size test (blue arrows), and the live/dead test (green arrow), they are sorted one at a time into tubes based on what antibodies are present on their surface (red arrows). Top panel shows cells from a coil, where we successfully identified and sorted 31 endothelial cells from a device that was inside a patient's aneurysm. Bottom panel shows cells taken from the femoral sheath, a device that is housed inside the patient's femoral artery. The latter cells serve as our internal controls.

Aim 2: Perform targeted transcriptome measurement of collected endothelial cells by single-cell RNA sequencing (RNA-Seq).

In order to perform next generation single-cell RNA sequencing, it was necessary to extract messenger RNA (mRNA) from the single cell samples we generated with high efficiency. This is very important as the amount of recoverable mRNA determines whether we can generate an adequate cDNA library to sequence the entire human genome. This step was performed using the SmartSeq Single Cell system (Fig 3). Through a series of steps, the mRNA from each endothelial cell is extracted, amplified into cDNA by polymerase chain reaction and subsequently purified (Fig 4) and validated (Fig 5).

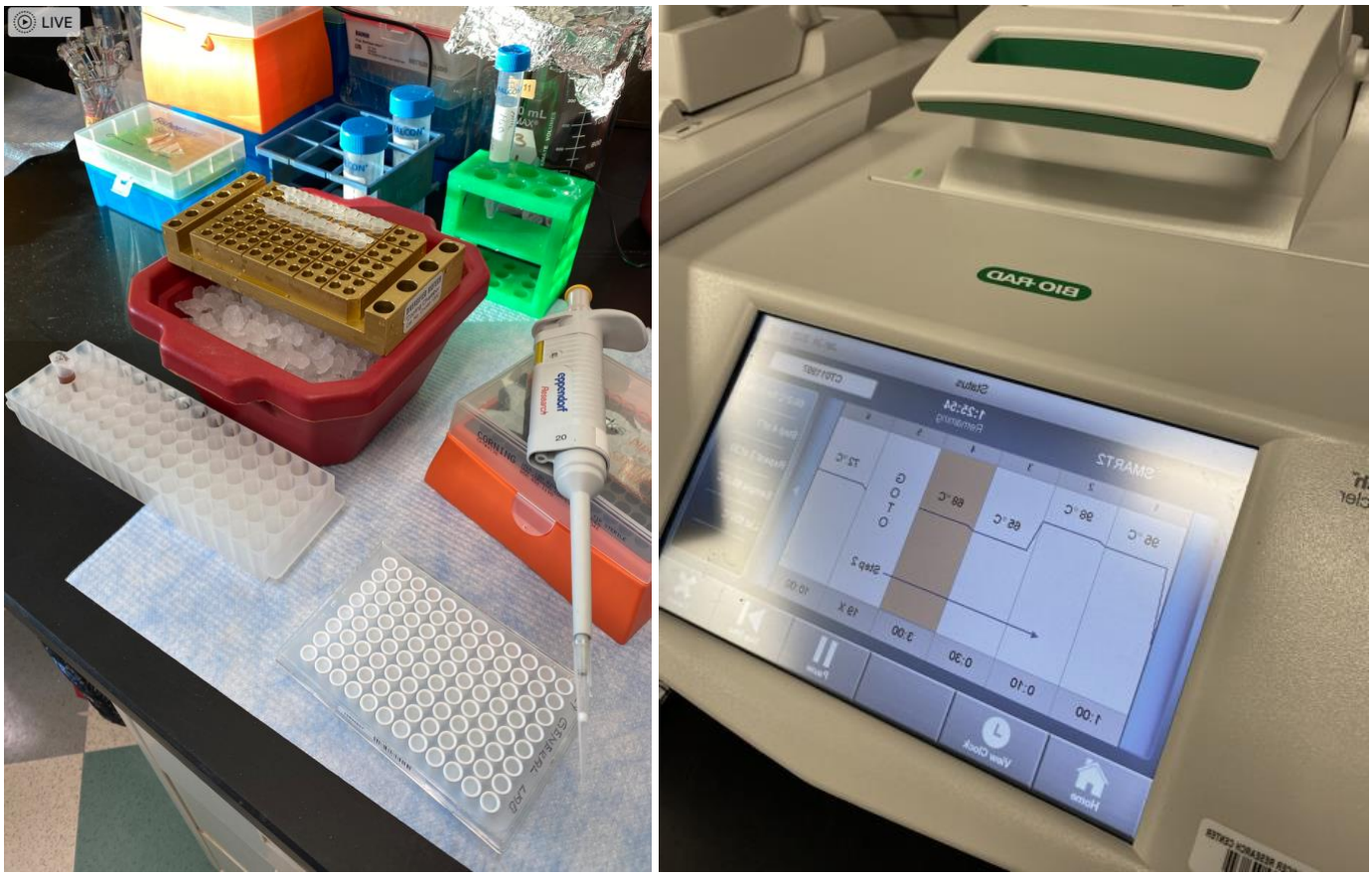

Fig 3. Lab bench set up for SmartSeq Single Cell RNA extraction (left). The extracted mRNA is amplified overnight by PCR into stabled double stranded cDNA (right).

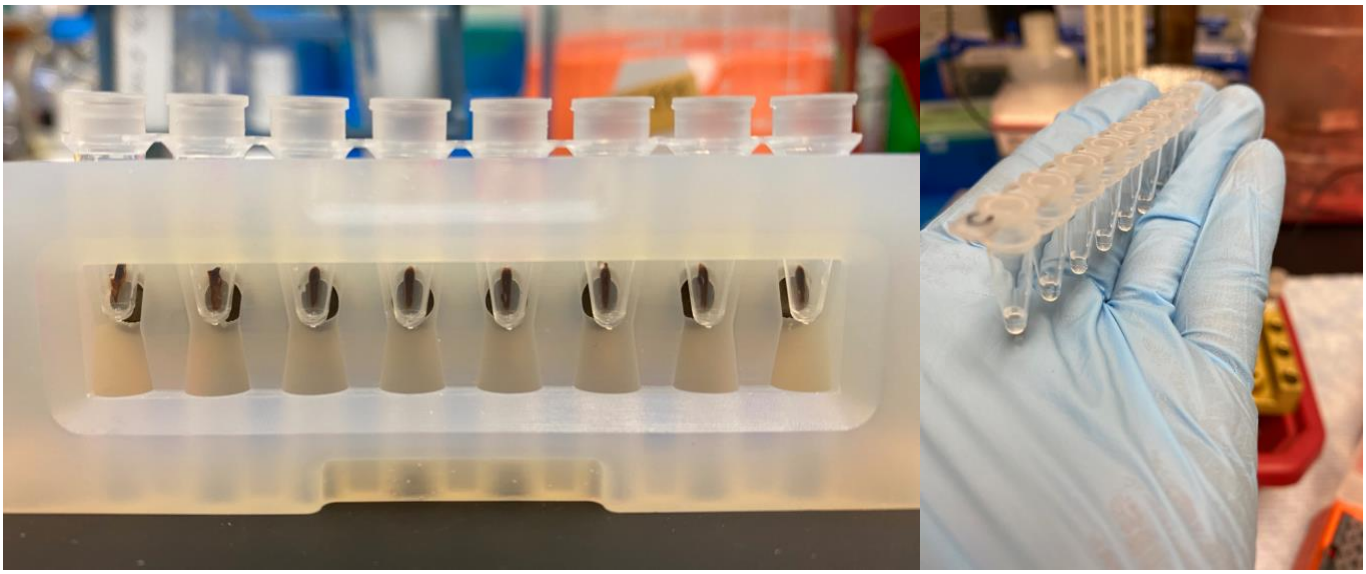

Fig 4. cDNA samples from 8 individual endothelial cells is purified using the AmPure Bead system (left). Note the dark opaque strands in the PCR tubes which contains the cDNA samples. Purified cDNA in clean PCR tubes which can be stored for several months at -80°C (right).

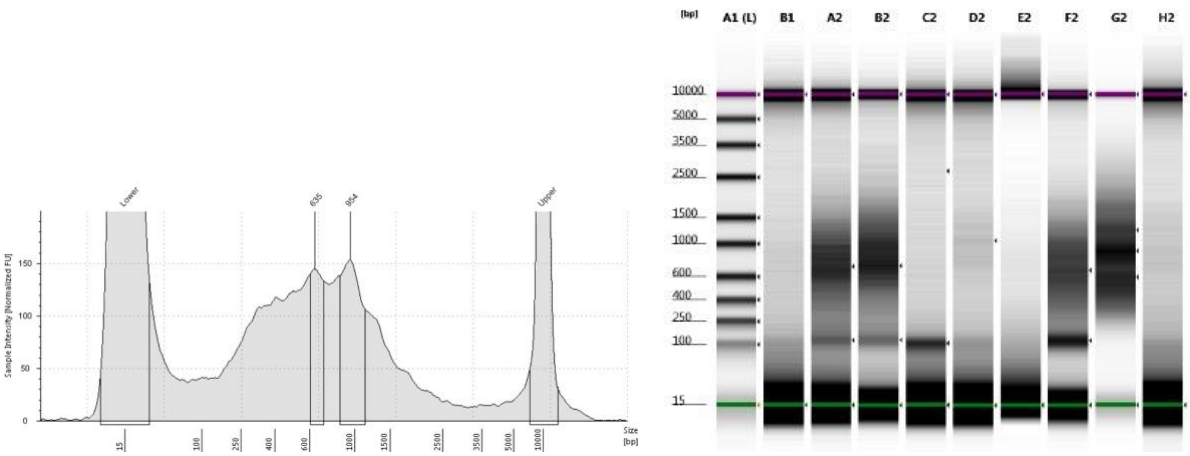

Fig 5. Validation of cDNA amplification by automated electrophoresis. Our cDNA samples demonstrated expected sized bands and appropriate concentration for cDNA library construction.

Impact of COVID-19 and going forward

Seattle was one of the first COVID-19 epicenters in the country, having reported the nation's first case and death in late January and February respectively. As part of the progressive and cohesive strategy by local and state authorities, there was a rapid escalation of social distancing and shelter in place measures starting in early March 2020. These restrictions have effectively contained the pandemic in our region; however the cessation of non-essential research by the University of Washington has completely curtailed our ability to collect samples and perform molecular biology experiments for the past 3 months.

These developments could not have come at a worse time for our research. By February 2020, we had completed optimization of coil collection, FACs and the single cell RNA extraction techniques and were in a position to move onto the next phase of our work and being larger scale cell collection, RNA extraction, cDNA library construction and data sequencing.

As of June 15th, we have a graduated restart to our research activities and our laboratory and core facilities will begin to come back online. We have also developed a partnership with the Allen Brain Institute here in Seattle, who will assist with aspects of the RNA extraction and sequencing work in order to expedite the progress of this project. Based on current projections, our objective is to achieve the stated research goals by the end of the 2020 or spring of 2021.