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My Laidlaw Journey (May 2019-Aug 2020)

This past year has been nothing short of fruitful given the opportunities that the partnership between The Laidlaw Foundation and University Scholars Programme (NUS) has given me. Before I reflect on my learnings this past year, I just wanted to thank everyone that has been part of this experience – The Laidlaw Foundation, Professor Kang Hway Chuan, Professor Low Boon Chuan and Miss Joanna Chua for giving me this opportunity, Dr Sudhakar Jha, Shreshtha Bhatia and James Lee for providing me with guidance during my research programme, Shane and Shamantha from Growthbeans for their insightful leadership workshops and, the team from Zhejiang University for making both exchange summer seminars possible.

1.0 The Leadership Aspect

A quick glance across the world during this COVID-19 pandemic highlights one thing above the rest – the importance of a good leader cannot be ignored. Countries with effective leadership have won the battle whilst countries with poor leadership continue to suffer. I used to be of the opinion that people can only be leaders if they are born with the traits that we associate with a leader, however, this process has taught me that while natural charisma is something innate, leadership skills can definitely be acquired. The way in which these skills are comprehended and applied by an individual is what lends diversity to leadership. Effective leadership looks different for everyone and there is no style that is necessarily better than the other.

1.1 Summer 2019 – Overseas Experiential Learning, Zhejiang University

My Laidlaw journey officially started in Summer 2019 when we went for the week long seminar on the topic of Strategic Leadership, Innovation and Society, organised by Zhejiang University. That week, albeit short, was eye-opening in many ways. The experiential programme covered concepts of future leadership and applied it to the more conventional domains of computer sciences, life sciences, medicine, innovation, management and the environment. I was especially interested in the lecture by Professor Xiaobo Wu of Zhejiang University who talked about the dynamics of innovation and how some Chinese companies specifically used their knowledge on exploiting the paradigm shift to become successful. The reason I found this intriguing was that I had not previously thought about the theoretical knowledge that went into making market decisions. It allowed me to appreciate the notion that successful leadership involves a blend of theoretical background and market experience to a large extent.

The seminars in the week amalgamated into a group presentation where we had to choose a particular domain and demonstrate our learnings. My group decided to combine our expertise in different fields namely politics, physics and biology to present a proposition to establish Life on another planet. The

process of doing this project together with classmates from the Chu Kochen Honors college was very interesting. It was really great that we got the chance to bounce off ideas one another especially since this project allowed everyone to chip in with their own field background knowledge. I still remember our brainstorming session in a small cosy Café behind the university where we were all so excited at the prospect of combining everyone's interests. The slight language barrier taught me to express my thoughts differently whilst retaining the gist of my point. This being said, this barrier did not hinder our progress at all since we were all very understanding of one another and appreciative of what each individual brought to the table. It also helped that everyone in my group wanted to keep the presentation light-hearted and fun which allowed us to flex our creative muscles to disguise our presentation as a short skit for everyone.

This exchange was also enriching from a cultural exchange perspective. Apart from the cultural trip to West Lake, there were other aspects of the Chinese culture that I learnt from my interactions with the people around me. For example, I was very intrigued by the cashless systems and advanced Artificial Intelligence technologies embedded in daily processes. I remember how shocked I was when someone behind me in a queue walked up to the cashier and paid for his groceries using facial recognition while I struggled to count my loose change.

1.2 Leadership Course by Growthbeans

This series of workshops by Growthbeans that spanned both summers allowed me to re-evaluate the kind of leader I wanted to be and provided me a platform to work on my personal growth. My key takeaways from this course were:

1. Learning how to give constructive feedback which is an essential skill for leaders to keep their team on the right track without dampening spirits . Another important aspect of this is active listening which is crucial if a leader wants to maintain a cooperative and cohesive team that values and embraces different perspectives.
2. Learning how to read non-verbal cues which can help leaders get a gauge of the emotions of their team without having to explicitly ask them about their feelings
3. Working on my confidence in making a coherent speech. After I told Shane and Shamantha that I often found myself talking in a roundabout way and repeating my points, they gave me ample opportunities to improve this during the workshop. I think that that definitely helped me as I am now a little better at making concise arguments that still hold water.
4. Learning the tips and tricks for maximising a networking session. I learnt the importance of crafting your story in such a way that appeals to the audience especially in a networking setting which is designed to talk to people and connect with them. For example, since my Laidlaw project is related to cancer, Shane and Shamantha taught me how I could use a personal approach to introduce my project

by perhaps starting by saying something like "We all know someone who has cancer... they may not be our immediate family member but could be someone's aunty or cousin." The point being to take the notion that cancer is prevalent and spinning it to give a personal touch to appeal to my listener's pathos.

Overall, I think that more than anything, this course allowed me to take a more active role in my own growth. I have become more aware of the areas that I need to work on and have been taking consistent steps to improve on them.

2.0 The Research Aspect

I spent summer 2019 also working on the ideation process for my project with my supervisor, Dr Jha. I held several meetings with his PhD students to discuss the implications of their findings on my project. Holding such conversations with them allowed me to gain an insight into the type of research that goes on in Dr Jha's lab as well as to build a rapport with the very people that were going to guide me in the lab come the following year. After much discussion, I finally settled on a project that I thought was interesting and that could be accomplished in 10 weeks.

2.1 Introduction to my project

R-loops are structures that form in the nucleic acid during transcription when nascent RNA strand invades the double-stranded DNA. These R-loops are different from the short RNA-DNA hybrids that form transiently during lagging-strand DNA synthesis. These instead span 100-2000 base pairs. While R-loops participate in many physiological processes, they are also a source of DNA damage since they are highly unstable and fragile. The most widely used method for R-loop mapping is DNA-RNA immunoprecipitation (DRIP)-sequencing which uses next-generation sequencing to map R-loops isolated by S9.6 Immunoprecipitation. Previous studies have identified multiple regions in the genome which form R-loop when TIP60 (HIV Tat interactive protein), a tumor suppressor is depleted for normal cells. Among them, 67 regions contain an APOBEC (Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) signature. Since R-loops cause DNA damage and given that the two hallmarks of cancer are high levels of DNA Damage and mutagenesis, the potential association between R-loops and cancer can be explored. This project aims to clone and express 10 selected sites out of the 67 identified R-loop regions into plasmids and using CRISPR-Cas9 technology to determine if introducing mutations into the genome using the Cas9 editing enzymes results in cancerous growth in cells.

2.2 The Research Experience

Summer 2020 unfortunately did not start smoothly given the closure of laboratories due to the COVID-19 pandemic. With a delay in the start of hands-on experiments, I tried to not let the time go to waste. Instead, I used the two weeks at home to plan a day-to-day schedule for my now limited time in the lab

as well as do a thorough literature review to re-fresh my knowledge on my research topic. This helped me once I started my work as I had a rough plan of what I had to do and a sound background to interpret my results. Additionally, I went ahead to order the necessary reagents since they took time to be delivered amidst the pandemic.

The original aim of this study was to clone and express the 67 identified R-loop regions into plasmids and to use CRISPR-Cas9 technology to determine if introducing mutations into the genome using Cas9 variants results in cancerous growth in cells. However, with the time crunch factor, it was necessary to reduce the 67 regions for testing by 85% to 10 regions instead.

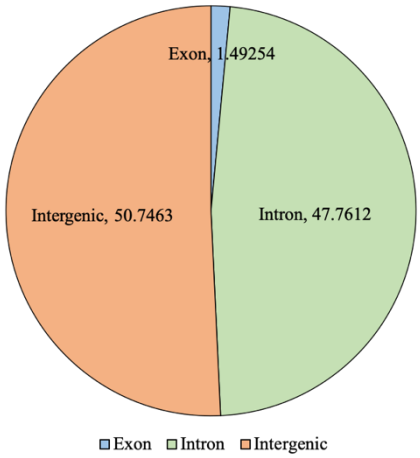

Figure 1: Distribution of 67 R-loop sites extracted from Shreshtha Bhatia's project

Selection of these 10 regions was not done at random. The following were 2 key criteria used to select 10 regions out of 67 for this study:

1. [Proportion of regions] According to the distribution of 67 regions in the genome, a similar proportion of the 10 regions should be used since the results gathered from these 10 regions act as a proxy for the remaining 57 regions. Hence, this project studied:
    a. 1 exonic region
    b. 5 intergenic regions
    c. 4 intronic regions

2. [Gene association] Since there was only 1 exon identified in the previous project, this was immediately selected to be included. The remaining 9 regions, consisting of 5 intergenic and 4 intronic regions, were selected on the basis of whether they were associated with a gene responsible for growth (since the basic definition of cancer is abnormal growth due to uncontrolled cell division) and if there was scientific evidence that a mutation in the associated gene resulted in a growth-related disease/cancer phenotype (see below).

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The table below summarizes the reasons for choosing all 10 regions as well as other relevant information.

No.	Annotation	Gene associated with the region	Reason for study selection
1	Exon	TTN-Antisense RNA 1	This gene encodes a non-coding RNA transcribed from the opposite strand to the titin gene (affiliated with the lncRNA class). Diseases associated with TTN-AS1 include lung adenocarcinoma and esophageal carcinoma.
2	Intron	Desmocollin 3	Altered expression of desmocollin 3 (DSC3), a member of the desmosomal cadherin family involved in mediating cell-cell adhesion, is found in various cancers such as lung and colorectal cancer. Studies have also shown that DSC3 expression is regulated by p53, a tumour suppressor gene.
3	Intron	SMARCAD1	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin. This gene plays a critical role in the restoration of heterochromatin organization and propagation of epigenetic patterns following DNA replication. Any mutation in this gene might result in improper packaging of the genome ("opening of the genome"), resulting in the expression of genes that were previously repressed. This may lead to uncontrolled growth. Studies have shown that SMARCAD1 is involved in breast cancer metastasis.
4	Intron	UBR5 antisense RNA 1	The Ubiquitin-Proteasome System (UPS) is an important regulator of cell signalling and proteostasis, which are essential to cellular processes. Hence, disruption of UPS is associated with cancer, amongst other diseases. E3 Ubiquitin ligases regulate key proteins commonly associated with proliferation and cell-cycle arrest (such as p53, p27, Cyclins, and NF-kB). The E3 ligase UBR5 is emerging as a key regulator of the UPS in cancer and development. UBR5 expression is deregulated in many cancer types and UBR5 is frequently mutated in mantle cell lymphoma. *UBR5 is rarely mutated in healthy somatic tissues, but is mutated and/or overexpressed in cancer.
5	Intron	RBMS3 antisense RNA 1	This gene encodes an RNA-binding protein that belongs to the c-myc oncogene single-strand binding protein family. These SSB proteins have been implicated in such diverse functions as DNA replication, gene transcription, cell cycle progression and apoptosis.
6	Intergenic	long intergenic non-protein coding RNA 901 (LINC00901)	Diseases associated with LINC00901 include Osteogenic Sarcoma and Gastric Cancer. Exact mechanism of action of this gene product is unknown, however, the presence of a TP53 responsible element upstream of their coding region suggests an involvement in the TP53 tumour suppressor gene signalling pathway.
7	Intergenic	AT-rich interaction domain 1B	Involved in transcriptional activation and repression of genes by chromatin remodelling (alteration of DNA-nucleosome topology). It is a component of SWI/SNF chromatin remodelling complexes that carry out key enzymatic activities, changing chromatin structure by altering DNA-histone contacts within a nucleosome in an ATP-dependent manner (similar reasoning to SMARCAD1)
8	Intergenic	Ras association domain family member 10	RASSF10 is a tumour suppressor gene which suppresses breast cancer growth by activating P53 signaling. Demethylation of the RASSF10 promoter is accompanied by the re-expression of RASSF10 in cancer cell lines.

9	Intergenic	Regulator of G-protein signalling 6 (RGS6)	G protein-coupled receptors (GPCRs) are involved in almost every physiological process, and a dysfunction in their signalling is linked to many human diseases. RGS proteins determine the magnitude and the duration of the GPCR signalling. Studies have shown RGS6 to play a critical role as a tumour suppressor and mediator of DNA damage signalling. It has associations with Glioma, Pancreatic cancer, Breast cancer, Bladder cancer, Ovarian cancer and lung cancer.
10	Intergenic	proteasome assembly chaperone 1 (PSMG1)	Proteasomes regulate cellular proliferation and apoptosis, both of which are dysregulated in cancer, by degrading intracellular proteins. The role of PSMG1 is to promote the assembly of the proteasome.

After site selection, I was ready to commence my project. There were three main steps to my project of which I was unable to get positive results for the first step in order to move on to the subsequent two steps. This initially caused me a lot of frustration before I decided to take the "failures" into my stride and use them as learning opportunities. Dr Jha always told me that we learn from our failures more than successes and often times, learning the "hard" way, as he puts it, is more rewarding. I can definitely attest to that since I learnt a lot about the scientific process of troubleshooting and trying repeatedly until something works. I would not have gained so much experience doing quality checks to see why my enzyme was not working properly if everything went smoothly the first time and to an extent, I am grateful for that opportunity. Dr Jha always guided me to think critically and encouraged me to blend theoretical knowledge with practical knowledge, in order to conduct more focused quality checks. While it was difficult to not have discussions with Dr Jha face to face due to COVID-19, his PhD students really played a huge role in guiding me in the lab especially since it was my first time working in a lab alone, without an instructor. For this, I am very thankful for their support. Additionally, despite the hardships faced, I am also glad that I was able to learn the different methodologies for each step in my protocol since I spent time optimising those processes before conducting the actual experiment.

3.0 Final Conclusions

This entire process has been nothing short of amazing. I have learnt so much about the research process from the start to the end. Although part of me is upset that I could not finish my project in its entirety, I have probably learnt a lot more in the process to figure out where things went wrong instead of following the protocol blindly. This journey has also taught me to adapt to unforeseen circumstances given the unprecedented pandemic – where I had to cut the breadth of my research due to the time crunch, not having face to face discussions with Dr Jha etc. More importantly, the Laidlaw journey has taught me so much about myself as a leader. Moving forward, my goal is put everything I have learnt into practice in order to make the most out of my takeaways from this experience.