General version of SoP

Zhou Ying zhouying_ch@qq.com

I have completed two Master's programs, specializing in neurodevelopment and bioinformatics, respectively. During the interim period between these two graduate programs, I also acquired two years of work experience at a hospital-based research platform. Through my research experience, I see the value in integrating wet and dry experiments for comprehensive solutions to complex biological challenges. Transitioning from technical skills to scientific inquiry, encounters with distressed patients in hospitals have spurred my passion to advance disease treatment. With expertise in bioinformatics and neurodevelopment, I aspire to delve into neural stem cell (NSC), brain tumor (like GBM), cancer stem cell (CSC), and related fields within a PhD program. My primary focus is on leveraging bioinformatics for novel insights, and validated them through biological experimentation. This statement of purpose is solely focused on presenting my personal interests and motivation for pursuing a PhD, particularly emphasizing the research aspect. For my personal story, please refer to the Personal Statement.

0.1 Previous research

During my undergraduate studies in bioengineering at Hunan Agricultural University, I gained a solid foundation in theoretical knowledge and experimental skills, exploring areas from computation to biology. This led me to pursue a Master's degree in neurodevelopment at Fudan University, where I engaged in projects focusing on stem cells in the cortex gyri of developmental ferrets and the classification of interneurons. These projects deepened my understanding of brain development and neuronal complexity. Interneuron classification project has been published and summarized on a poster, it is also my thesis, while the cortical gyrus development project is under submission (poster). Additionally, the details of these two projects can be found in team meeting slide decks. I developed an interest in bioinformatics during my final year of studies, leading me to a position as a clinical data analyst at a hospital, where I evaluate variant prioritization tools for rare diseases, utilizing an entropy-weighted ensemble method to improve molecular diagnoses. After submission a related paper (has published, poster can be found here), I pursued a second Master's in bioinformatics at Harbin Medical University, where I focused on transcriptome, epigenome, and translatome sequencing analysis (e.g., single-cell and bulk RNA-seq, ChIP-seq, CUT&TAG, Ribo-seq). After reviewing literature and past experiences, and discussing with my team, I decided to compare fetal brain and brain tumor cell transcriptomes at the single-cell level (poster). Detailed information is available in my team meeting slide decks. This diverse experience has equipped me with essential wet and dry experimental skills for future doctoral studies.

0.2 Proposed research

My expertise lies in quantitatively analyzing neuron feature proliferation, the activities of neural progenitors within cortical structures using microscope image-based techniques, and various sequencing analysis methods. I am particularly interested in NSC and its differentiation, especially those associated with tumor cells that exhibit remarkable proliferation capabilities similar to stem cells, posing a threat to health. Tumors present specific cellular abnormalities at multiple levels of molecular biology, necessitating computational skills to unveil complex mechanisms and identify treatment targets. Upon encountering literature discussing glioblastoma stem cell (GSC) as a crucial factor in recurrence [1], with some likening them to NSC, I developed a keen interest and delved into various papers on NSC.

In the realm of NSC, it's imperative to distinguish between fetal (fNSC) and adult (aNSC) stem cell. fNSC exhibit widespread inside-out multi-layer cellular structures within the fetal brain, whereas aNSC are primarily located in the dormant state within the subgranular zone (SGZ) and subventricular zone (SVZ), becoming activated in response to particular stimuli, such as injury [2]. Regarding GBM as an adult glioma, three hypotheses emerge regarding their origin: 1) mature cells adopting stem-like properties, 2) abnormal differentiation of activated aNSC in response to stimuli, or 3) a combination of the two, wherein mature cells trigger an inflammatory response, activating quiescent aNSC, leading to abnormal differentiation into tumor cells (personal hypothesis). GBMs have been observed to invade the ventricular region, with studies indicating cells in the SVZ secreting factors attracting GBCs towards them [3]. Despite these findings, the precise relationship between GBCs and aNSC remains elusive, sparking my personal curiosity for further investigation. Moreover, a subset of aNSC expressing markers similar to radial glial cells (RGCs) reported to exist in GBM [4]. aNSC also shares morphological similarities with truncated radial glia (tRG), a subtype of RGCs. Additionally, tRGs are known to generate a significant portion of ependymal cells [5], sharing an origin with aNSC [6]. My recent research also observed heightened activation of tRG pathways in GBM cells, suggesting a potential link between tRGs, aNSC, and GBCs, which could offer new insights into GBM treatment.

In summary, I am curious in four questions: 1) Can tRG transition into dormant aNSC residing in the SVZ of adult animals? 2) Is there a mechanism linking aNSC to the generation of GSC? 3) Do GBCs also enter a quiescent state similar to aNSC, and if so, what molecules are involved and how might this relate to recurrence? 4) Can the mechanism for reverting active aNSC back to quiescence and inhibit GBM progression?

Hypothesis of tRG-aNSC lineage. Despite limited research on the molecular regulation of the tRG-aNSC lineage relationship, understanding it could reveal crucial factors activating quiescent tRG switches to become dormant aNSC. This insight might impact GBM progression by targeting quiescent genes in GBCs. To investigate, I propose marking tRG in mouse embryo brains using viral vectors or electroporation, a technique I've employed previously. Upon animal maturation, we'll confirm tRG to aNSC transition using aNSC' markers or other identification tests. If validated, we'll collect marked cells over development time and sequence them to uncover crucial molecular changes along the tRG-aNSC trajectory. For sequencing, scRNA-seq offers detailed insights if finances permit, leveraging my experience in data analysis. Alternatively, bulk RNA-seq combined with published datasets can provide valuable information. Moreover, phenotypic profiling experiments (such as marked tRG shape description, cell activities recording in time-lapse) will complement molecular analyses to establish correlations between cellular phenotypes and molecules over time. This proposal will address the first question regarding the relationship of tRG and aNSC. I have expertise in bulk/single-cell RNA-seq analysis, virus injection/electroporation, cell culture, time-lapse imaging, IHC, and other techniques. However, I lack experience in aNSC study. Thus, for doctoral research of this direction, I seek a team with interests and expertise in NSC, particularly aNSC, with the necessary experimental conditions.

Exploring the Link Between aNSC and GSC Generation. Based on my review of literature and insights from my current project comparing scRNA-seq data of GBCs and fNSC, I hypothesize that certain stimuli trigger injury responses, activating dormant aNSC. However, errors in this process may lead to malignant transformations. To validate this hypothesis, I aim to investigate the association between GSC and activated aNSC at a molecular level. One approach involves inducing injury or inflammation in mouse brains to activate aNSC, followed by sequencing and comparison with untreated mouse cells. If GSC originating from aNSC or their progenies are identified, further analyses will focus on differential gene expression and molecular mechanisms. Additionally, comparisons between the differentiated lineage of aNSC and GBCs will be made, potentially through developmental trajectory analysis of scRNA-seq data. Furthermore, exploring whether the normal mechanisms controlling aNSC activation post-injury repair can be leveraged to suppress GBC progression is of interest. This could involve identifying key genes involved in aNSC activation or dormancy maintenance and manipulating them via viral vectors in GSC, followed by in vivo observation of tumor phenotypes upon transplantation into mice. Overall, I aim to unravel the pathways underlying the transformation of aNSC into GSC and their implications for GBM progression. This proposal will research the second and forth above question. I also have expertise in relevant techniques. I suppose that team experienced in NSC, GBM, or both, with corresponding experimental conditions, would be ideal for my doctoral study executing this proposal.

Hypothesis of some GSC in a quiescent state similar to aNSC. GSC have been implicated in recurrence following chemotherapy, radiotherapy, and other treatments [1]. Considering the reported relationship between GBCs and aNSC [3], I hypothesize that GSC may adopt a dormant state similar to aNSC, utilizing their mechanisms to protect themselves and survive treatment. To investigate this, methods similar to those proposed in the second proposal will be employed: transplanting GBM into mice, administering drug or radiotherapy to induce recurrence, and isolating GSC before and after treatment for sequencing. These GSC will then be compared with both activated and quiescent aNSC to elucidate their molecular profiles. Subsequently, differential analysis of molecular data between GSC associated with activated and quiescent aNSC will be performed. Identification of key factors or mechanisms promoting GSC transition into a quiescent state may lead to their impairment and subsequent validation through mouse models. Such mechanisms hold promise for application alongside classical treatments to mitigate recurrence and resistance.

Other application study when accessed some fundamental results. I participated in an application project aimed at optimizing algorithms for variant interpretation within a hospital setting. Through this experience, I gained insight into how molecular diagnostic techniques can be connected to fundamental research outcomes to enhance clinical practices or studies. A pivotal aspect of this endeavor was the development of a knowledge database that stores correlations between molecules and diseases or cellular phenotypes, along with computational tools to assist in molecular diagnostic efficiency. Consequently, could I identify significant molecules associated with specific phenotypes or mechanisms conducive to molecular diagnostics, I am keen on exploring further into tool development or applied research in this domain.

After completing my PhD, I aim to secure a position in a university or research institution, dedicated to advancing scientific research in the biomedical field. My ultimate goal is to contribute my skills and expertise towards biomedical progress, whether as an investigator, technician, teacher, or in any relevant capacity.

[1] X. Lan, D. J. Jörg, F. M. G. Cavalli,et al. Fate mapping of human glioblastoma reveals an invariant stem cell hierarchy. *Nature*, 549(7671):227–232, Sept. 2017.

[2] N. Urbán, I. M. Blomfield, and F. Guillemot. Quiescence of Adult Mammalian Neural Stem Cells: A Highly Regulated Rest. *Neuron*, 104(5):834–848, Dec. 2019.

[3] E. Y. Qin, D. D. Cooper, K. L. Abbott, J. Lennon, et al. Neural Precursor-Derived Pleiotrophin Mediates Subventricular Zone Invasion by Glioma. *Cell*, 170(5):845–859.e19, Aug. 2017.

[4] R. Wang, R. Sharma, X. Shen, A. M. Laughney,et al. Adult Human Glioblastomas Harbor Radial Glia-like Cells. *Stem Cell Reports*, 14(2):338–350, Feb. 2020.

[5] M. Bilgic, Q. Wu, T. Suetsugu, Y. Tsunekawa, et al. Single cell transcriptomics of ferrets reveal a common temporal pattern of progenitors in brain development of gyrencephalic mammals, May 2022.

[6] G. Ortiz-Álvarez, M. Daclin, A. Shihavuddin, P. Lansade, et al. Adult Neural Stem Cells and Multiciliated Ependymal Cells Share a Common Lineage Regulated by the Geminin Family Members. *Neuron*, 102(1):159–172.e7, Apr. 2019.