

**RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)**

Completion Report on Funded Project

Project start date: 1 September 2012
Project completion date: 31 August 2017

1. Project Title:

Stem Cell Strategy for Nervous System Disorders

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the whole project period
Project Coordinator	Nancy Ip Chair Professor	Life Science / HKUST	12 hrs
Deputy Project Coordinator	Zhenguo Wu Professor	Life Science / HKUST	12 hrs
Co-Principal Investigator(s)	Kenny Chung Associate Professor	Life Science / HKUST	10 hrs
	Sookja K. Chung Professor	Biomedical Sciences / HKU	10 hrs
	Kai Liu Associate Professor	Life Science / HKUST	10 hrs
	Robert Qi Professor	Life Science / HKUST	10 hrs
	Jianan Qu Professor	Electronic and Computer Engineering / HKUST	10 hrs
	Karl Tsim Chair Professor	Life Science / HKUST	10 hrs
	Zilong Wen Professor	Life Science / HKUST	10 hrs
	Yung Hou Wong Chair Professor	Life Science / HKUST	10 hrs

	Jun Xia Professor	Life Science / HKUST	10 hrs
	Wing Ho Yung Professor	Biomedical Sciences / CUHK	10 hrs
	Mingjie Zhang Chair Professor	Life Science / HKUST	10 hrs
Co-investigator(s)	Tom Cheung Assistant Professor	Life Science / HKUST	6 hrs
	Bo Feng Assistant Professor	Biomedical Sciences / CUHK	6 hrs
	Jufang He Professor	Biomedical Sciences / CityU	6 hrs
	Xuhui Huang Associate Professor	Chemistry / HKUST	6 hrs
	Randy Y.C. Poon Professor	Life Science / HKUST	6 hrs
	Penger Tong Chair Professor	Physics / HKUST	6 hrs
	Weimin Zhong Associate Professor	Molecular, Cellular and Developmental Biology / Yale U	6 hrs
	Zhongjun Zhou Professor	Biomedical Sciences / HKU	6 hrs
Collaborators	Kwong-yau Chan Consultant	Neurosurgery / Kwong Wah Hospital	N/A
	Bruce R. Conklin Professor	Medical Genetics and Molecular Pharmacology / UCSF	N/A
	Xiang Gao Professor	Model Animal Research Center / Nanjing U	N/A
	Yukiko Gotoh Professor	Molecular and Cellular Biosciences / Tokyo U	N/A
	Haifan Lin Professor	Cell Biology / Yale U	N/A

	Guo-li Ming Professor	Neuroscience / U Penn (w.e.f. Mar 2017)	N/A
	Thomas Rando Professor	Neurology and Neurological Sciences / Stanford U	N/A
	Hongjun Song Professor	Neuroscience / U Penn (w.e.f. Mar 2017)	N/A
	Yi Sun Associate Professor	Molecular and Medical Pharmacology / UCLA	N/A

Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. Basic Research		
1.1 Elucidate the regulatory mechanism that controls the balance between proliferation and differentiation of neural progenitor cells	100%	Completed
1.2 Investigate the intrinsic machinery that regulates the differentiation and maturation of newborn neurons, and their eventual integration into neural networks	100%	Completed
2. Functional Validation		
2.1 Use cell-based models to validate the therapeutic efficacies of signaling pathways/molecules	100%	Completed
2.2 Use animal models to validate the therapeutic efficacies of signaling pathways/molecules in animal models	100%	Completed
3. Translational Research		
3.1 Identify agents (small molecules/TCM extracts) that possess neurogenic activities	100%	Completed

* Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.

** Please provide reasons for significantly slower rate of progress than originally planned.

6. **Research Highlights and Outputs**

(Maximum 20 A4 pages for sections 6 to 11, excluding any appendices and attachments)

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.)

This TRS project is uniquely positioned to leverage the team members' basic research strengths in the areas of molecular neuroscience and stem cell biology for knowledge-based drug discovery for nervous system disorders. In the past five years, under the guidance of a panel of distinguished advisors together with the recommendations of the UGC TRS Sub-committee, the TRS team has successfully achieved all of its designated goals and made significant accomplishments in terms of both basic research and drug discovery. We have selected the following research accomplishments to illustrate the overall success of the TRS project.

(1) Significant contributions to the understanding of the regulatory mechanisms that control neurogenesis

Our research group has made significant contributions to the understanding of the molecular mechanisms that regulate the coordinated balance between the proliferation and differentiation of neural stem cells (NSCs). Imbalance of this precisely regulated process leads to various neurological disorders including microcephaly (insufficient neuron production), macrocephaly (excessive neuron production), and schizophrenia. Thus, characterizing the molecular mechanisms that control this process might provide essential insights into the pathophysiology of various neurological disorders and identify potential therapeutic targets.

Unlike most stem cells, asymmetric cell division (ACD) is a unique property of NSCs that allows them to undergo self-renewal to maintain the NSC pool and simultaneously generate neurons and glia. ACD is precisely regulated such that the proliferation of NSCs and production of new neurons and glia are properly maintained. Disrupting this delicate balance results in the depletion of NSCs or uncontrollable cell proliferation, which could lead to cancer. ACD is regulated by several polarity complexes that act cooperatively to set up the apical--basal polarity of dividing cells and arrange the asymmetric distribution of signaling molecules (e.g., RNA, proteins, and lipids). The activity of polarity complexes determines cell fates such as proliferation, differentiation, and cell migration. There are three major polarity complexes—the Crb, Par, and Scrib complexes—which comprise Crb/PALS1/PATJ, Par3/Par6/aPKC, and Scrib/Lgl/DLG proteins, respectively. The Par complex is coupled to the NuMA–LGN (or AGS)–G α i complex via the Insc protein to orchestrate mitotic spindle orientation, which affects cell fate determination during ACD. Furthermore, LGN recruits DLG protein, which also couples to the mitotic spindle. Determining the structures of different members of the protein complexes and understanding the relationships of their intra- and inter-molecular interactions during ACD can help decipher the molecular events and functions of the different protein complexes involved in neurogenesis. In this TRS project, we have revealed the intricate interactions of the members of these complexes. Our findings (as described below) have significantly contributed to the fundamental understanding of the molecular interaction of polarity complexes and their regulation of ACD.

We have elucidated how the membrane-associated guanylate kinases (MAGUKs), which contain multiple domains, exert a high degree of binding specificity with their binding partners. PALS1 is a MAGUK that contains the characteristic PDZ–SH3–GK modules, and interacts closely and specifically with Crb in the Crb complex. It was originally thought that PALS1 only utilizes its

PDZ domain to bind to the C-tail of Crb. However, our study revealed that all three constituent modules of PDZ–SH3–GK in PALS1 are required for the binding of the protein to Crb's tail. This is an important discovery that expands our understanding of the synergistic mechanism of proteins utilizing multiple distinct binding modules to interact with their target peptides for protein complex assembly (Li et al., *Proc Natl Acad Sci USA* 111:17444-9, 2014). This finding was highlighted in *Proc Natl Acad Sci USA* 111:17350-1, 2014. Furthermore, we demonstrated that an actin-binding protein, Moesin, competes with PALS1 for binding to the C-tail of Crb. Phosphorylation of the Crb C-tail by aPKC greatly affects binding between Crb and Moesin, but not with PALS1 (Wei et al., *J Biol Chem* 290:11384-92, 2015). These results collectively suggest that the activity of the Crb complex is regulated by aPKC-dependent phosphorylation.

Precise regulation of microtubule dynamics in the developing neurites of newborn neurons is critical for axon specification during neuronal polarization. Our studies demonstrated that Par3 of the Par polarity complex directly regulates microtubule stability and organization via its N-terminal domain. The N-terminal domain activity of Par3 can be suppressed by its C-terminal portion via an intramolecular interaction (Chen et al., *Dev Cell* 24:26-40, 2013). Of note, the intermolecular oligomerization of Par3 can relieve the intramolecular interaction and thereby promote microtubule bundling and stabilization. We further elucidated the structures of the Par3 N-terminal domain as well as the filaments formed by the polymerization of Par3 N-terminal domain (Zhang et al., *Structure* 21:997-1006, 2013). We found that disruption of Par3 N-terminal domain self-association significantly impacts the Par3-mediated epithelial polarization. This finding demonstrates that Par3 has direct role in microtubule organization during neuronal polarization. Meanwhile, we found that the Par complex is coupled to the spindle orientation complex NuMA–LGN (or AGS)–Gai via Insc protein to orchestrate the mitotic spindle orientation during ACD.

We further dissected how the intra- and inter-molecular interactions of the crucial protein, LGN, regulate the apical macro-polarity complexes comprising the Par and spindle orientation complexes (Pan et al., *Structure* 21:1007-17, 2013). LGN is a scaffold protein that functions at an important crossroads by interacting with multiple protein players; it connects the Par complex, Gai·GDP, and NuMA. It also interfaces with microtubules via its N-terminus or central domain. Of note, the scaffolding activity of LGN requires its release from the auto-inhibited conformation through the binding of Gai via its C-terminal GoLoco (GL) motifs. Our results suggest that external cues might signal to the cells via G-proteins, which in turn breaks the auto-inhibitory mode of LGN, allowing LGN to orchestrate mitotic spindle dynamics through its interaction with its upstream and downstream protein partners.

On the other hand, LGN interacts with the spindle fibers via NuMA or DLG, which is a MAGUK. While MAGUK proteins share a signature guanylate kinase (GK) domain that binds to many target proteins, the currently known mode of action of MAGUK GK is via binding to phosphorylated short peptides (e.g., binding to LGN). Our groundbreaking study showed that the GK domain of DLG MAGUK binds to an unphosphorylated domain of the kinesin motor KIF13B, with high specificity and affinity (Zhu et al., *Structure* 24:1876-1885, 2016). Thus, the structure of DLG4 GK in complex with KIF13B revealed the molecular mechanism governing this atypical GK/target recognition, thus expanding our understanding of the action mechanisms of MAGUK family proteins. Furthermore, these findings provide insights into the role of DLG in the regulation of ACD.

(2) Significant contributions to the understanding of the differentiation and integration of newborn neurons

In order for newborn neurons to integrate into the existing neural circuitry, they must migrate to their final destinations and make synaptic contacts with existing neurons. The whole process,

including synaptic integration and subsequent survival of these newborn neurons, is precisely controlled by various signaling pathways, including signal transduction from the cell-surface receptors to their intracellular targets (as described below). We have made several seminal discoveries on the regulatory mechanisms involving specific signaling molecules such as Cdk5, $\alpha 2$ -chimaerin, MC4R-dependent signaling, and neuroligins.

We have demonstrated that the precise regulation of a serine/threonine kinase cyclin-dependent kinase 5 (Cdk5) is critical for proper neuronal migration, dendritic arborisation, and synapse development. Specifically, we showed that Cdk5 regulates neuronal migration by modulating the activity of another serine/threonine kinase, Mst3. The Cdk5-dependent regulation of Mst3 in neuronal migration is mediated through the negative modulation of RhoA, a critical organizer of the actin cytoskeleton. These results suggest that a coordinated and orchestrated signaling cascade is critical for signal transduction to the intracellular cytoskeletal network to subsequently regulate neuronal morphology during neurogenesis. A report of this study was published in Tang et al. *J Neurosci* 34:7425-36, 2014. Furthermore, we showed that Cdk5-mediated transcriptional control is a key mechanism in neuronal activity-dependent dendrite development. During membrane depolarization, Cdk5 accumulates in the nucleus to regulate the expression of a specific subset of genes, including the brain-derived neurotrophic factor (BDNF), which is a critical neurotrophic factor for dendritic growth. Importantly, the transcriptional regulatory effect of Cdk5 is mediated through the phosphorylation of methyl-CpG-binding protein 2, a key transcriptional repressor whose mutation is associated with Rett syndrome, a mental disorder. The Cdk5-dependent phosphorylation of methyl-CpG-binding protein 2 is critical for controlling its interaction with the promoter of *bdnf*, and hence the subsequent transcription of *bdnf*. These findings highlight the importance of the phosphorylation-dependent control of transcription regulation during the development of newborn neurons; a report of this study was published in Liang et al. *J Neurosci* 35:15127-34, 2015.

More recently, we have identified the differential roles of p39 and p35, the activators of Cdk5, in dendritic development. We showed that p39-dependent Cdk5 activity is specifically required for dendritic development. In addition, we found that Cdk5 activity is required for the proper dendritic development of hippocampal neurons. Of note, depletion of p39 but not p35 impairs the dendritic development of cortical and hippocampal neurons owing to decreased dendritic length and arborization. Furthermore, we examined the signaling pathways involved in p39-knockout neurons through transcriptomic and phosphoproteomic analysis. We found that Cdk5/p39 modulates some proteins related to actin and microtubule dynamics (manuscript under preparation). These findings collectively demonstrate that the precise regulation of Cdk5 activity is critical for different processes of neurogenesis, including neuronal migration and dendrite development. Recently, we have also demonstrated that Cdk5-dependent phosphorylation is critical for the regulation of excitatory synapse formation through modulating the trafficking of postsynaptic proteins. We showed that Cdk5 phosphorylates liprin- $\alpha 1$ in an activity-dependent manner and regulates liprin- $\alpha 1$ functions at the postsynaptic sites. Importantly, the Cdk5-phosphorylation of liprin- $\alpha 1$ regulates the synaptic translocation of PSD-95, a key postsynaptic scaffold protein that regulates excitatory synapse development. Interestingly, the phosphorylation status of liprin- $\alpha 1$ in the mouse visual cortex is precisely regulated by eye opening. Our findings collectively indicate that Cdk5 activity and its phosphorylation of liprin- $\alpha 1$ are involved in activity-dependent synapse development (Huang et al. *Proc Natl Acad Sci USA* 114:E6992-E7001, 2017). In summary, the Cdk5-dependent phosphorylation cascades enable the transduction of signals from extracellular cues to regulate various intracellular processes including cytoskeletal dynamics, transcriptional machinery, and protein trafficking to coordinate the multiple processes of neurogenesis.

While adult neurogenesis is a life-long process critical for the self-renewal of neurons, our

understanding of its underlying molecular and cellular bases is limited. Therefore, we studied the role of a Rho GTPase-activating protein (GAP), $\alpha 2$ -chimaerin, in adult neurogenesis. The adult mouse hippocampus highly expresses $\alpha 2$ -chimaerin, and $\alpha 2$ -chimaerin-knockout mice exhibit decreased adult hippocampal neurogenesis compared to controls. Moreover, we found that conditional deletion of $\alpha 2$ -chimaerin from adult NSCs/NPCs results in the premature differentiation of NSCs, which leads to the depletion of the NSC pool and impaired neurogenesis in the long term. Single-cell RNA-sequencing analysis of the hippocampus of $\alpha 2$ -chimaerin conditional-knockout mice revealed specific loss of a subpopulation of early NSCs, indicating precocious depletion of NSCs. We further showed that this premature differentiation of NSCs is due to an imbalance between the asymmetric and symmetric division of adult NSCs. Deletion of $\alpha 2$ -chimaerin in adult-born neurons resulted in impaired dendritic development, suggesting poor neuronal integration. Finally, we found that $\alpha 2$ -chimaerin conditional-knockout mice exhibited increased anxiety/depression-like behaviors under chronic restraint stress (manuscript under preparation). Thus, our findings revealed the functional roles of Rho GAPs in adult neurogenesis and provided insights into the molecular mechanisms underlying the etiology of adult neurogenesis-related disorders such as major depression and anxiety.

The integration of newborn neurons into neural circuitry is tightly regulated. We have demonstrated that precise activation of a G protein-coupled receptor (GPCR), melanocortin 4 receptor (MC4R), is critical for the regulation of hippocampal synaptic plasticity. Melanocortin receptors and their ligands, pro-opiomelanocortin (POMC)-derived peptides, which were originally known to regulate energy metabolism, are implicated in learning and memory. However, the functional roles of POMC/melanocortin receptors in hippocampal synaptic plasticity, and hence learning and memory, are unclear. We were the first to demonstrate that MC4R activation enhances the phosphorylation of the AMPA receptor subunit GluA1, which is critical for the surface expression of AMPA receptors. In addition, we found that activation of melanocortin/MC4R pathways in the hippocampus enhances the dendritic spine density of pyramidal neurons and hippocampal synaptic plasticity, and thereby long-term potentiation (Shen et al. *J Neurosci* 33:464-72, 2013). We subsequently confirmed the functional roles of MC4R in hippocampal synaptic plasticity by demonstrating that stimulation of neuronal activity increases the *in vivo* secretion of melanocortin in the hippocampus through mapping the POMC circuitry in the mouse hippocampus (Shen et al. *Cell Rep.* 17:1819-1831, 2016). To dissect the roles of the GPCR effectors in hippocampal synaptic plasticity, we adopted the “Designer Receptors Exclusively Activated by Designer Drugs” (DREADD) technology to study how activation of Gas-coupled receptors in the hippocampus regulates learning and memory. We found that GsD activation by clozapine-*N*-oxide (CNO) enhances GluA1 phosphorylation and hippocampal long-term potentiation. Moreover, activation of GsD in the hippocampus by different doses of CNO resulted in different impacts on the contextual memory of mice.

Furthermore, we have revealed the molecular control of synaptogenesis. Neuroligins (NL) are cell adhesion proteins whose roles in the initiation of synaptogenesis are well characterized; deficiencies in their functions lead to autism. Thus, it is critical to identify proteins that interact with NLs and elucidate their functional roles in synaptogenesis. We found that an E3 ubiquitin ligase, Itch, is an NL2-associated protein. The association of Itch and NL2 results in the ubiquitination and degradation of NL2 protein. While NL2 plays a critical role in the formation of inhibitory synapses, overexpression of Itch in neurons significantly reduces the number of inhibitory synapses. Blocking NL2–Itch interaction abolished the effect of Itch in reducing inhibitory synapses, indicating that the Itch–NL2 complex has a regulatory role in mediating the formation of inhibitory synapses (manuscript under review).

(3) Association of impaired neurogenesis with neurological disorders

Proper production of excitatory and inhibitory neurons is critical for the functional integrity of the neocortex, whereas dysregulation of neuronal production leads to neurodevelopmental disorders, including microcephaly, autism, and schizophrenia. While genetic studies have provided insights into the involvement of specific proteins that are associated with these disorders (e.g., DISC1 in schizophrenia and CDK5RAP2 in microcephaly), the functional roles of these proteins remain unclear. Hence, we have examined the function of these proteins in neurogenesis and provided experimental evidence to illustrate how their dysregulated functions contribute to the pathophysiology of the corresponding diseases. These findings can provide important insights into the pathological basis of neurodevelopmental disorders. Also, appropriate animal models are critical for examining the functional roles of specific proteins during the pathogenesis of neurodevelopmental disorders. In this regard, manipulations of target proteins that modulate neurogenesis may give rise to invaluable animal models.

We have elucidated the molecular role of a protein CDK5RAP2, a centrosomal protein whose mutations cause microcephaly, in the regulation of ACD. CDK5RAP2 stimulates the microtubule nucleation activity of γ -tubulin ring complex (γ TuRC), which is required for its centrosomal attachment. Depletion of CDK5RAP2 impairs astral microtubule formation and attenuates spindle orientation, pointing to a possible role in ACD. Using the γ TuRC-binding domain of CDK5RAP2, we purified γ TuRC and identified Nme7 and DNA polymerase δ catalytic subunit (PolD1) as two novel associated proteins. While Nme7 and PolD1 are not required for γ TuRC assembly, we demonstrated that Nme7 promotes the microtubule-nucleating activity of γ TuRC and PolD1 binds indirectly to CDK5RAP2 through γ TuRC. The Pol δ holoenzyme comprises the catalytic subunit PolD1 and three other subunits. We observed that the other subunits of Pol δ were not present in isolated γ TuRC, suggesting that γ TuRC is associated with free PolD1. Overexpression of PolD1 interfered with microtubule nucleation on centrosomes and the Golgi complex, whereas PolD1 inhibited microtubule nucleation induced by CDK5RAP2. Thus, our findings provide insights into the molecular and functional organization of γ TuRC. The study on Nme7 was published in Liu et al. *Mol Biol Cell* 25:2017-25, 2014.

Altered embryonic neurogenesis is associated with various neurodevelopmental disorders. For example, the brains of autistic patients exhibit excessive neuron production. However, the molecular or cellular basis underlying the etiology of autism is unclear. Therefore, we characterized the functional consequence of impaired embryonic neurogenesis. We showed that overproduction of upper-layer neurons in the neocortex during early development leads to autism-like features in mice. Our previous study revealed that the expression level of Axin, a scaffold protein, is critical for the precise control of neuron production during early brain development in a Cdk5-dependent manner by regulating the population of intermediate progenitors. To examine the functional consequence of neuron overproduction during embryonic neurogenesis, we stabilized Axin expression in the developing brain by injecting XAV939 (an Axin stabilizer) and observed that the upper-layer neurons were overproduced in the neocortex. Furthermore, we found that the increased production of upper-layer neurons in the mice perturbs the development of dendrites and spines of excitatory neurons and alters the laminar distribution of interneurons. These mice exhibit dysregulated excitatory and inhibitory synaptic connection and balance as well as behavioural abnormalities resembling those of human autism (Fang et al. *Cell Rep* 9:1635-43, 2014). This finding provides insights into the molecular and cellular basis underlying autism.

We have also investigated the role of DISC1 (disrupted-in-schizophrenia 1) in the division of NSCs and determined its contribution to schizophrenia (Ye et al. *Neuron* 96:1041-1054, 2017). Mutations of DISC1 are associated with major psychiatric disorders. However, despite the hundreds of reported DISC1-binding proteins, it is unclear how DISC1 structurally interacts with its protein

partner(s) during human brain development. First, we solved the high-resolution structure of DISC1 C-terminal tail in complex with its binding partner, Ndel1, which is a key component that regulates microtubules. Mechanistically, we found that DISC1 regulates Ndel1's kinetochore attachment but not its centrosome localization during mitotic cell division. Functionally, we found that disrupting DISC1–Ndel1 complex formation prolongs mitotic length and interferes with cell-cycle progression in human cells, thereby causing cell-cycle deficits of radial glial cells in the embryonic mouse cortex and human forebrain organoids. We observed similar deficits in the organoids derived from induced pluripotent stem cells (iPSCs) with a DISC1 mutation that disrupts its interaction with Ndel1 from a patient with schizophrenia. Thus, our study uncovered a new action mechanism for DISC1 based on its structure, which has implications for how genetic insults could contribute to psychiatric disorders.

Neuronal loss occurs and is often irreversible after nerve injury, which leads to functional impairments. Here, we demonstrated experimental therapeutic interventions capable of stimulating regeneration and functional recovery of injured axons. Nerve injury downregulates the protein expression of mammalian target of rapamycin (mTOR), a regulator of protein translation critical for regrowth. The phosphatase PTEN (phosphatase and tensin homolog) is a negative regulator of mTOR. Also, nerve injury upregulates suppressor of cytokine signaling 3 (SOCS3), which suppresses axonal regeneration. Deleting both PTEN and SOCS3 in retinal ganglion cells in a pre-chiasm optic nerve lesion model promotes their axons to re-innervate and form new functional synapses with neurons in the suprachiasmatic nucleus. Our results demonstrated that boosting the intrinsic growth capacity of injured neurons promotes functional axonal re-innervation (Li et al. *Neurobiol Dis* 73:366-76, 2015). In another study, using a mouse spinal cord injury model, we modulated PTEN/mTOR signaling in adult corticospinal motor neurons after injury. Deleting PTEN not only promotes sprouting of uninjured corticospinal tract axons, but also enables the regenerated axons to cross the lesion, even when the intervention is administered up to 1 year post-injury. Our results considerably extend the window of opportunity for regenerating corticospinal tract axons severed in spinal cord injuries in mice (Du et al. *J Neurosci* 35:9754-63, 2015). We also studied the effects of neuronal activity on axon regeneration. Overexpression of the light-sensing pigment melanopsin in retinas of mice enhances neuronal firing of retinal ganglion cells and promotes axonal regeneration after crushing of the optic nerve through activating mTOR signalling. We also stimulated neuronal activity through a chemogenetic approach by overexpressing DREADD-Gq and activating Gq signaling (downstream of melanopsin). Activation of DREADD-Gq signalling by daily administration of CNO resulted in a significant increase in axonal growth. Our findings indicate that modulating neuronal activity through GPCRs stimulates axonal regeneration after CNS injuries (Li et al. *Proc Natl Acad Sci USA*. 113:1937-42, 2016). We are now employing a stem cell-based strategy to treat nerve injury. Our studies so far have confirmed that transplanted NSPCs from E14 spinal cords can survive and differentiate into neurons within 4 weeks of transplantation into the injured spinal cord.

(4) Establishing NSC platforms, animal models, and new technological platforms

Patient-derived cellular systems can be used to study disease-associated factors such as genetic factors that cannot be accurately mimicked in animal disease models. The generation of iPSC-derived neural cells from normal individuals and patients is a promising approach to study different complex diseases. In this study, we have established different neural stem cell-based platforms including human iPSCs. Technologies based on iPSCs are invaluable because human-derived neural cells are critical for studying physiological functions and disease mechanisms. Additionally, we have established new animal models for the study of pathological basis of neurogenesis-related diseases. With the advances of *in vivo* imaging and electrophysiological

technologies, we have also developed methods to track and monitor the functional integration of the stem cell-derived neural cells into the host neuronal circuitry, thus enabling evaluation of their contribution towards functional improvement of the animals. Some of the technologies and platforms established herein also form the basis of future proposed work.

Over the course of this project, we established different NSC (neural stem cell)-based platforms, including human iPSCs, and developed protocols for the differentiation of specific neural cell types. Specifically, we established standard protocols for the generation of cortical neurons from human iPSCs. First, we directly induced iPSCs into neuronal lineage by dual inhibition of SMAD signaling. The differentiation of human NSCs was confirmed by the expression of NSC markers, nestin and Sox2. The differentiated neurons were then characterized by the expression of markers for deep-layer (CTIP2+) neurons and upper-layer (Brn2+) neurons, as well as astrocytes (GFAP+). We showed that neurons differentiated in this manner were able to form a functional neuronal network that exhibits electrophysiological properties. Successful establishment of the iPSC-derived neuronal system enables the study of disease mechanisms, identification of new molecular targets, and evaluation of drug candidates. This laid the technical foundation for the further establishment of platforms to generate iPSCs-derived neural cells from both healthy people and diseased patients. These can be used to probe the pathological mechanisms of neurodegenerative diseases and as cell models for drug screening. Notably, a new TRS project has been awarded to us to build upon these well-developed platforms and protocols, and further study the role of genetic risk factors in Alzheimer's disease using the iPSC-derived neural cell system.

Human iPSCs also hold great promise for treating neural injury and neurodegenerative diseases. Thus, we have studied the survival and characterized the functions of implanted iPSC-derived human NSCs in rat brains. To investigate their therapeutic potential, we first developed iPSCs derived from epithelial-like cells in human urine (Wang et al. *Nat Methods* 10:84-9, 2013). We then used a standard procedure by converting iPSCs into induced NSCs (iNSCs) through dual inhibition of Smad signalling. The iNSCs are highly neurogenic, and the iNSC-derived neurons show typical neuronal morphology, exhibit specific neuronal gene expression, and are capable of firing action potentials. The matured neurons also express 5-HT₂ receptors, which allow serotonin to modulate the postsynaptic GABA receptor currents. Furthermore, these human neurons can integrate into pre-existing rodent neural circuitry when cultured with rat primary cortical neurons.

We also transplanted iNSCs into the cerebral cortex of rats and found that the transplanted cells survive for up to 6 months. At four months, the human cells differentiate and migrate to the neighbouring structures in the rat brain. To further study whether these transplanted neurons can connect with the host cells to form a functional neuronal network, we developed an iPSC cell line expressing channelrhodopsin protein. We examined whether these channelrhodopsin-expressing cells can send outputs to and receive inputs from the host brain cells upon transplantation. We also trained the rats to use optic stimulation as a cue for behavioural tasks, such as fear conditioning.

Identification of the signals that enhance functional integration of the transplanted iNSCs into the existing circuits is critical. We demonstrated that cholecystokinin (CCK) – a neuropeptide can facilitate neuronal plasticity (Li et al. *Cell Res* 24:307-30, 2014). Local infusion of CCK into the brains of rats induced plastic changes of the cortical circuit, which enabled the cortical neurons to potentiate their responses. Furthermore, CCK infusion potentiated the synaptic strength between neuronal connections. We are undertaking research to investigate whether CCK can enhance neuronal network formation between the human iNSC-derived neurons and primary rat neurons, and evaluate its ability to facilitate the functional integrations of the neural stem cells.

In addition to the stem cell-based models, we have also established various animal models, to examine the roles of specific signaling pathways and evaluate the therapeutic efficacies of specific

intervention approaches. Specifically, to characterize the roles of Axin and Epac2, two proteins with critical functions in neuronal signaling pathways, we established two mouse models – Axin overexpressing mice and mice with abolished Epac2 expression – to study the effect of Axin and Epac signalling on neurogenesis and stress-induced depression.

Serotonin reuptake inhibitors (SSRI), represented by Prozac, are widely used for treatment of depression; their action is proposed to be mediated by activating cAMP response element-binding protein (CREB)/protein kinase A (PKA) signalling and increasing the expression of a neurotrophic factor, BDNF. However, the involvement of specific signalling regulators that mediate the actions of drugs are unclear. Epac2 is a cAMP sensor protein, which is directly activated by cAMP and acts as a guanine nucleotide exchange factor to activate Rap small GTPase. Whether Epac2 is the potential downstream target of SSRI/cAMP in mood regulation remains unclear. Here, we showed that Epac2^{-/-} mice exhibit a wide range of behavioural defects associated with mood disorders, including anxiety and depression with learning and memory deficits in contextual and cued fear-conditioning tests. Interestingly, the gene encoding for Epac2 is associated with cognitive decline in Chinese Alzheimer's disease patients, suggesting that Epac2 signalling is involved in neurodegeneration. To further understand the role of Epac2 in SSRI/serotonin/cAMP-involved mood disorders, we demonstrated that treatment of Epac2^{-/-} mice with Prozac alleviates the alteration in open field behaviour and impaired hippocampal neurogenesis. Taken together, modulation of Epac2 functions may be a potential therapeutic approach for mood disorders (Zhou et al. *Transl Psychiatry*. 6:e881, 2016).

Other than mood disorders, enhancing neurogenesis may help to restore neuronal functions in neurodegenerative diseases. Degeneration of dopaminergic neurons in the midbrain is one of the major pathological hallmarks of Parkinson's disease. Thus, to better understand the pathological basis of Parkinson's disease, we generated and characterized various mouse models for study of the disease, including aldose reductase (AR)-knockout mice. AR regulates dopamine synthesis and the detoxification of reactive dopamine metabolite. In Parkinson's disease patients, the level of AR is significantly lower in the cerebellum. After treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an agent which can induce Parkinsonism, AR^{-/-} mice showed more severe behavioural deficits and brain damage in comparison with the wildtype mice. The AR^{-/-} mouse brains also showed signs of increased oxidative stress. These results are consistent with the role of AR in detoxification in dopaminergic neurons. Unexpectedly, AR^{-/-} mice showed significant elevation in dopamine in the midbrain, highlighting that AR-deficient mice can act as a good Parkinson's disease model to screen for drug candidates that protect dopaminergic neurons against neurotoxic metabolites in Parkinson's disease. We also established another transgenic mouse model, with the conditional deletion of CR6-interacting factor-1 (Crif1) in dopaminergic neurons (DATCrif1^{-/-}). DATCrif1^{-/-} mice have a shortened life span with progressive degeneration of substantia nigrostriatal dopaminergic neurons starting from postnatal week 5. They show an increased accumulation of α -synuclein with Lewy bodies in the substantia nigrostriatal region and a significant reduction in dopamine. Furthermore, they suffer from locomotion disability with obvious tremor. Administration of levo-DOPA (a dopamine agonist) attenuated the Parkinson's disease-like behavioral defects in these DATCrif1^{-/-} mice. In summary, DATCrif1-deficient mice act as a good model for understanding the pathogenesis of Parkinson's disease and can be used for developing strategies for therapeutic treatment, particularly on the functional restoration of dopaminergic neurons.

To study the functional integration of newborn neurons or transplanted neurons into mouse brains, we have established new technological platforms to facilitate deep brain imaging and *in vivo* electrophysiological recordings. First, an *in vivo* extracellular recording, adopted from Plexon Inc., was established. Here, we used multi-electrode arrays implanted into a target brain area to record

the activities of specific neuronal populations, as well as local field potential, in conscious freely moving rodents. Using this platform, recordings were made from the primary motor cortex in hemi-Parkinsonian animals during deep brain stimulation. Second, an *in vivo* patch-clamp recording was set up in head-constrained animals to study membrane excitability and synaptic transmission. Specifically, this system was employed to investigate alterations in synaptic connections in a Parkinson's disease model. Third, a multiphoton microscopic system was set up for use in conjunction with micro-optics (e.g. thick glass plugs) for high-resolution and large field-of-view two-photon imaging of deep tissue (>1.5 mm). The system can capture images of neuronal structures and large ensembles of neuronal activities based on fluorophores and functional calcium indicators, respectively. We set up optogenetics by expressing light-sensitive rhodopsins/halo-rhodopsins in specific neuronal populations together with light guide implantations to optically manipulate the activities and interrogate the functions of these neurons. Optogenetics was employed to investigate the impact of dopamine depletion on cognitive flexibility under Parkinson's disease. Head-mounted microendoscopy imaging adopted from Inscopix Inc. was also used to record the activity of large numbers of neurons in freely moving animals using calcium imaging. The electrical activity of a subpopulation of neurons and astrocytes was observed and distinguished by expressing cell-type specific calcium sensors. Lastly, we adopted a procedure called CLARITY to convert brain tissue from opaque into an optically transparent and permeable hydrogel-hybridized form, which makes it possible to obtain high-resolution information regarding anatomical structures, long-range cellular projections, and neuronal connectivity. CLARITY was combined with light sheet fluorescence microscopy to investigate the newly discovered hippocampal POMPC circuitry.

Furthermore, we have established new technological platforms to facilitate *in vivo* single cell labelling and cell lineage tracing. Cell fate mapping and lineage tracing are both highly significant means of understanding the developmental origins of various biological tissues. They require labelling of individual cells and tracing the development of all their progeny. Conventional approaches to cell labelling suffer due to low spatial resolution and are unsuitable for long-term cell tracing due to degradation of the intracellular fluorescent labelling molecules. Thus, we developed an infrared laser-evoked gene operator heat-shock microscopy to achieve precise single-cell labelling in zebrafish *in vivo*. Heat shock-mediated expression of transgenes was induced in targeted single cells through near-infrared laser radiation. A unique fluorescent thermometry technique was used to measure the temperature rise in zebrafish tissues induced by infrared laser heat shock. Efficient single-cell labelling was demonstrated in multiple types of cells in zebrafish, including myocytes, hematopoietic cells, and neurons.

(5) Identifying small molecules that possess neurogenic activities

Reduced adult neurogenesis in the hippocampus has been implicated in animal models of depression as well as in patients with major depressive disorder. Thus, screening for neurogenic compounds might help to develop therapeutic interventions for the related disorders. We have applied both target-based approaches and phenotypic screening for the identification of neurogenic agents.

Target-based approaches for the identification of neurogenic agents were focused on two kinases, Cdk5 and EphA4. We demonstrated that the aberrant activation of these two kinases result in the deregulation of different cellular processes in neurogenesis. Furthermore, aberrant activation of Cdk5 is associated with different neurological diseases, including Alzheimer's, Parkinson's, and Huntington's diseases. Likewise, hyperactivation of EphA4 has been implicated in various diseases, including cancer, Alzheimer's disease, spinal cord injury, and amyotrophic lateral sclerosis. Thus, we aimed to identify small molecules that can inhibit the activity of the kinases.

To identify small molecule inhibitors of Cdk5, we first conducted a molecular dynamics simulation to obtain a large number of different conformations of Cdk5. These conformations were then subjected to ensemble *in silico* drug screening – a structure-based computer aided drug design process to identify candidate small molecules with high accuracy. The inhibitory activity of the candidate small molecules was then evaluated through biochemical and cellular assays. Alongside, we aimed to identify agents, specifically extracts, derived from several well-known traditional Chinese medicine (TCM) herbs that can enhance neuronal differentiation by phenotypic screening. Using this approach, we have identified two TCM-derived compounds which exhibited non-competitive inhibition of Cdk5. Subsequently, we identified a novel drug binding site on Cdk5, which can explain the ATP-non-competitive inhibition of Cdk5 exerted by the two TCM-derived compounds. We have subsequently conducted molecular dynamics to examine the property of the binding site and proposed residues that are critical for the binding of the kinase and the small molecules. Further mutagenesis studies were undertaken to verify molecular interactions between Cdk5 and candidate compounds.

To identify EphA4 inhibitors, a number of computational approaches were utilized to search for compounds that bind to the extracellular ligand-binding domain of EphA4. For example, we conducted a virtual screening of an in-house TCM database and FDA-approved drugs through molecular docking between the extracellular domain of EphA4 and candidate compounds. A small molecule derived from TCM, rhynchophylline, was found to bind tightly to EphA4 using *in vitro* biochemical analysis. We next demonstrated that rhynchophylline effectively blocked EphA4-dependent signalling in hippocampal neurons, as revealed by the inhibition of tyrosine phosphorylation of the receptor and the decrease of EphA4-dependent growth cone collapse. Oral administration of rhynchophylline reduced EphA4 activity effectively in the hippocampus of a neurodegenerative mouse model. Furthermore, rhynchophylline administration restored the impaired long-term potentiation in the transgenic Alzheimer's disease mice, suggesting that blockade of EphA4 signalling can improve the AD-associated synaptic dysfunctions (Fu et al. *Proc Natl Acad Sci USA* 111:9959-64, 2014). Other than improving the impairment of synaptic plasticity, we also demonstrated that rhynchophylline administration increased adult hippocampal neurogenesis in aged mice as well as in the AD transgenic mice.

A major challenge of drug development is the toxicity of lead compounds. Thus, repurposing already-approved drugs for other indications presents a viable solution for resolving this challenge. Several drugs have been successfully repurposed through the use of both *in silico* and *in vitro/vivo* approaches. We have identified five FDA-approved drugs — ergoloid, cyproheptadine, nilotinib, abiraterone, and retapamulin — as potential inhibitors of EphA4 by using an integrated approach combining virtual screening with biochemical and cellular assays. We screened a database of FDA-approved drugs against the ligand-binding domain of EphA4 using a molecular docking method. Twenty-two candidate drugs were selected based on their docking energy with EphA4, and their inhibitory activity towards EphA4 was then evaluated. Five drugs were shown to exhibit EphA4 inhibitory activity as revealed by the decrease of EphA4 clustering induced by ephrin-A in cultured primary neurons. Specifically, the kinase inhibitor nilotinib exhibits EphA4 inhibitory activity in the cellular context. The compound inhibited the binding of EphA4 and ephrin-A at micromolar scale in a dosage-dependent manner, and inhibited the activity of EphA4 (indicated by the decrease of EphA4 tyrosine phosphorylation) and EphA4-dependent growth cone collapse in cultured hippocampal neurons. Together, we provide experimental evidence to support the repurposing of FDA-approved drugs as EphA4 inhibitors, which may provide an alternative fast-track approach for identifying and developing new treatments for different diseases, particularly Alzheimer's disease (Gu et al. *Sci Rep* 8:7377, 2018). In addition, we have established a docking method to screen for small molecules that interact with the ligand-binding domain of

EphA4 and characterized in detail the possibilities of docking conformations of the EphA4 inhibitors screened in-house. The findings from this study have led to the licensing of the screening and development of EphA4 inhibitors to a pharmaceutical company.

The neurohormone, melatonin, has been demonstrated to exert a neurogenic effect in a human neural stem cell model (ENStem-A cells). Given that many natural products contain melatonin, we embarked on the screening of TCM extracts for their ability to induce differentiation of ENStem-A cells. Ethanol extracts of *Radix Rehmanniae*, *Radix Astragalus*, and *Rhizoma Chuanxiong* significantly enhanced neuronal differentiation in various neural progenitor cell lines. Dose response analysis confirmed the neurogenic potentials of these candidate extracts. A high-throughput screening on a panel of TCM-derived compounds further revealed that 7-hydroxy-6-methoxy-2-methyl-2H-isoquinolin-1-one is a selective melatonergic ligand. A distinctive feature of this compound is the lack of a free alkylamide side chain but an *N*-methylamide moiety confined in the isoquinolinone scaffold. Subsequently, a series of substituted isoquinolinones were synthesized and characterized. Structure-activity relationship analysis revealed that substituted isoquinolinones bearing a 3-methoxybenzyloxy group at C5, C6, or C7 position, respectively, conferred effective binding and selectivity toward the MT₂ melatonin receptor (*PLoS One* 9:e113638, 2014). Further fine-tuning of the isoquinolinone-based compounds might yield melatonin receptor subtype-specific therapeutic agents with neurogenic activity.

Depression and anxiety disorders are emerging health problems that affect millions of people worldwide, with limited treatment options. Impaired adult hippocampal neurogenesis, which is a regulator of mood control and mediator of antidepressant efficacy, is implicated in the pathogenesis of anxiety and depression. In contrast, increasing adult hippocampal neurogenesis can buffer stress response and is required for the beneficial effects of several antidepressants. The scaffold protein Axin is a key regulator for modulating the protein levels of GSK-3 β and β -catenin, two critical molecular players in adult hippocampal neurogenesis. We demonstrated that increasing the expression of Axin by administration of the Axin stabilizer XAV939 enhances embryonic neurogenesis and alters social interaction behaviours (Fang et al. *Cell Rep* 9:1635-43, 2014). We further showed that stabilizing Axin in the adult mouse brains by XAV939 injection increases adult hippocampal neurogenesis and exerts an antidepressant effect. Specifically, treating adult mice with XAV939 increases the amplification and differentiation of adult neural progenitor cells in the hippocampus under both normal and chronic stress conditions. Moreover, the XAV939-treated mice exhibit more mature granule neurons in the hippocampus, suggesting that the increased immature neurons induced by XAV939 injection can successfully mature into granule neurons and integrate into the dentate gyrus circuitry. Functionally, XAV939 injection in mice ameliorates depression-like behaviours induced by chronic restraint stress. Thus, our study demonstrates an important role of XAV939/Axin in adult hippocampal neurogenesis and increasing Axin expression provides a potential therapeutic approach for mood-related disorders (manuscript under review).

(6) Strategic collaborations with renowned academic and industry partners.

The TRS team has strived hard to build ties with both academic collaborators and the biopharmaceutical industry in order to strengthen and enhance both the basic research and translational aspects of the TRS. Close ties were developed with the prestigious Karolinska Institutet (KI) during the course of this project. Team members participated in the “2014 Karolinska Institutet-Hong Kong Regenerative Medicine Retreat” held on 23-24 October 2014 at HKU. The retreat brought together investigators from KI and Hong Kong to foster future collaborations on regenerative medicine in the academic, clinical, and industrial settings. Joint efforts between researchers from KI and Hong Kong will foster future collaborations on regenerative medicine that can lead to new insights and innovations.

The team has also developed a strategic relationship with the Morningside Group, a Boston-based biotechnology company for the commercial development of some of the findings that arose from this TRS pertaining to the discovery of EphA4-inhibiting agents. Patent applications have been filed for these research findings and the technologies were successfully licensed to the Morningside Group for further development along with HKUST team.

In 2010, the first and only State Key Laboratory was established at HKUST (The State Key Laboratory of Molecular Neuroscience). The SKL Scheme identifies accomplished scientists undertaking pioneering research to support China's technological development. Hence, this national standing represents national recognition of HKUST's unremitting efforts and excellence in conducting neuroscience research and development. The research activities conducted during this TRS project further enhanced this status. Specifically, regular scientific exchanges with experts at the Institute of Neuroscience of the Chinese Academy of Sciences (our partner State Key Laboratory in Shanghai) helped to enrich and promote academic excellence in Hong Kong. During a recent site visit to the SKL at HKUST, the Ministry of Science and Technology delegation applauded the efforts and scientific output of the research activities undertaken as part of this TRS project.

Additionally, a Center for Stem Cell Research (CSCR) was established at HKUST under this TRS project. The mission of the CSCR is to position HKUST as a prominent center for stem cell R&D while stimulating interest from leading biopharmaceutical companies. The long term goals of this center are to position Hong Kong as a regional center for neural stem cell R&D and significantly boost Hong Kong's biopharmaceutical industry. The CSCR will expand on some of the work initiated from this TRS project, and has successfully received seed funding from HKUST.

6.2 What was the added value of the TRS funding, rather than standard project grant funding?

(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

Our TRS-funded program is a research-intensive collaborative venture, necessitating a concerted, multidisciplinary approach, which has required long-term funding commitment to harness expertise across various disciplines of basic and translational research. A very strong level of synergy has been achieved among team members and our collaborators to devise and conduct innovative studies as well as establish new technologies. For example, the DISC1 study resulted from an extensive collaboration between the Zhang group and TRS's external collaborators, Profs. Hongjun Song and Guo-li Ming. Meanwhile, the deep brain imaging platform was established from the partnership between Prof. Nancy Ip's lab in the Division of Life Science and Prof. Jianan Qu's group in the Physics Department. Additionally, the single-cell RNA-seq and lineage-tracing analysis platforms for adult neurogenesis in the adult hippocampus resulted from a collaboration between Profs. Tom Cheung and Nancy Ip. In these cases, the scale of expertise required and the extent of research conducted span beyond what could have been undertaken and achieved in a normal GRF or CRF grant.

The TRS funding grant also enables annual meetings to be held, through which we have invited eminent scientists from Hong Kong, mainland, and abroad, thus providing networking and collaboration opportunities between team members and external collaborators. Furthermore, the depth and length of our TRS-funded program has served to provide outstanding training and personal development opportunities to postgraduate students and junior researchers, beyond what is possible through other grants. Here, they have participated in advanced cutting-edge science, received mentoring from accomplished scientists, gained research experience in a highly-interactive and collaborative atmosphere, learned to work with other research groups, as well as had the valuable opportunity to generate and present scientific findings.

6.3 If the project has not met its original objectives, why?

The TRS team has achieved all of the original objectives.

6.4 (a) Peer-reviewed journal publication(s) arising directly from this project:

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

Over the course of the TRS project, the breakthrough research undertaken by the team has resulted in a large volume of publications in prestigious journals such as *Dev Cell*, *Neuron*, *Cell Report*, *Nature Comm*, *J Am Chem Soc*, *PNAS*, *JAMA Neurol*, *J Neurosci*, and many others in journals such as *Stem Cell Res*, *PLoS One*, *J Biol Chem*, *Cell Signal*, and *Planta Med*. To date, the TRS team has published 186 research papers in areas including neural development and functions, neuronal signalling mechanism, and structure-function relationship of neuro-proteins. Please refer to **Appendix 1** for the complete list of journal publications.

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered: *(Please attach a copy of each conference abstract)*

The TRS team has actively promoted their breakthrough findings through international symposium and conferences such as the prestigious Gordon Research Conference, the Annual Meeting of the Society for Neuroscience, Cold Spring Harbour Conference, and the EMBO Conference. Team members have also been invited to delivery plenary or symposium lectures at international conferences and workshops. A total of 171 TRS-related presentations have been given by our members. Please refer to **Appendix 2** for the list of conference abstracts.

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

Conference abstracts are often bound by a strict word limit and as a result, the acknowledgement part in some of the abstracts was automatically removed during submission.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

Collaborations, including intra- and inter-institutional as well as local and international, are greatly emphasized in our program. These collaborations have resulted in a number of joint publications (**Appendix 1**). For example, the study published in *PNAS* 2016 entitled "Promoting axon regeneration in the adult CNS by modulation of the melanopsin/GPCR signaling" was the result of a collaborative effort between Profs. Kai Liu and Yung Hou Wong, who have strong expertise in axon regeneration and G-protein signaling, respectively. Collaboration was also undertaken between Profs. Yung Hou Wong and Mingjie Zhang, resulting in a joint publication in *Mol Cell Biochem*. 2015. Among the inter-intuitional collaborations, Profs. Nancy Y. Ip and Kai Liu (from HKUST) collaborated with Profs. Liwen Jiang (not TRS member) and Wing Ho Yung (from CUHK), with the resulting work published in *Cell Rep*. 2014. Additionally, Profs. Kai Liu, Nancy Y. Ip and Yung Hou Wong (from HKUST) and Prof. Wing Ho Yung (from CUHK)

collaborated on work that resulted in a publication in *Neurobiol Dis.* 2014. Other notable collaborations include Profs. Mingjie Zhang and Randy Poon (from HKUST) with the TRS external collaborators Profs. Hongjun Song and Guo-li Ming (both from U Penn), who combined their efforts to elucidate the role of DISC1/Ndel1 and DISC1/ATF4 in adult neurogenesis; and Profs. Jufang He (City U) and Jun Xia (HKUST), who collaborated to study the role of galanin-containing neurons in auditory cortex activity and short term memory. Prof. Jufang He (City U) also collaborated with Profs. Duanqing Pei and Guangjin Pan (both from Guangzhou Institutes of Biomedicine and Health, CAS, not TRS members) to establish the co-culturing of primary rat cortical neurons and human iPSC-derived neurons. Other past and on-going collaborations are listed in **Appendix 3**.

Furthermore, we had a number of productive meetings with our international collaborators – Profs. Guo-li Ming, Hongjun Song (both from U Penn) and Prof. Yi Sun (UCLA). Prof. Thomas Rando from Stanford University also met with us a few times at HKUST to give us valuable advice and discuss about possible future collaboration. We also extended our partnerships with international organizations. Close ties were developed with the prestigious Karolinska Institutet (KI). Team members participated in the “2014 Karolinska Institutet-Hong Kong Regenerative Medicine Retreat” held on 23-24 October 2014 at HKU. The retreat brought together investigators from KI and Hong Kong to foster future collaborations on regenerative medicine in the academic, clinical, and industrial settings. Prof. Tom Cheung is currently collaborating with Profs. Maria Kasper and Rickard Sandberg of KI to understand stem cell population in different tissues using the single cell sequencing approach. Joint efforts between researchers from KI and Hong Kong will foster future collaborations on regenerative medicine that can lead to new insights and innovations.

Collectively, the success of the TRS project has greatly enhanced the notion of inter-institutional collaborations among the team members. The TRS has enabled members from different universities to work synergistically towards a common objective, and the complementary research skills and knowledge have opened up new avenues of research, strengthened research competencies, established new technologies, and built a strong portfolio of drug leads. This outcome will foster further cross-institutional research and drive initiatives that foster research collaborations. For example, Prof. Penger Tong’s lab in Physics and Prof. Robert Qi’s lab in Life Science at HKUST have submitted a joint proposal to RGC to further investigate the volumetric and viscoelastic properties of mitotic cells and their response to external mechanical cues.

The collaboration between Nancy Y. Ip and Xuhui Huang (HKUST), using molecular docking with a library of small molecules originating from traditional Chinese medicine, resulted in the discovery of EphA4 inhibitors. One of the EphA4 inhibitors identified, rhynchophylline, exhibited beneficial effects in transgenic mouse models of Alzheimer’s disease (Fu et al., 2014, PNAS), and a series of derivatives based on rhynchophylline were subsequently generated. A similar approach was then conducted using a FDA approved drug library, which led to the identification of additional EphA4 inhibitors (Gu et al., 2018, Scientific Reports) that can potentially be repurposed for Alzheimer's disease treatment. Patent applications have been filed in multiple jurisdictions for these research findings, which have been secured (US9629830, ZL201480039831.8, JP2016-526434) or are pending (EP20140826591; HK16108023.6; CA2918340, PCT/IB2018/053315). Moreover, the technologies have been successfully licensed to the Morningside Group, a Boston-based company supporting biotechnology development. In the third quarter of 2018, its subsidiary company applied for funding from the University and Industrial Collaborative Program of Innovation and Technology Commission to initiate preclinical development of EphA4 inhibitors with the HKUST team.

6.6 Research students trained (registration/awards):

By undertaking this project, the cross-institutional team has actively engaged in pioneering studies utilizing advanced technologies, which has resulted in numerous invaluable training opportunities (and technical and academic exchanges) at the forefront of stem cell research for postgraduate students. A total of 76 students received valuable hands-on technical experience through this project, which will contribute to the formation of a critical mass of expertise in advanced neural regenerative medicine in Hong Kong. Please refer to **Appendix 4** for the list of postgraduate students trained. Moreover, a large number of undergraduate students also benefitted from this TRS as they received training from the participating laboratories through their Final Year Projects or the University Research Opportunities Programme.

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

Patents granted or under examination:

The direct output of the TRS project is numerous with significant therapeutic and commercial appeal (particularly to biopharmaceutical companies interested in acquiring emerging technology for further development). This is especially true for novel compounds that exhibit neuroprotective features that make them good candidates as therapeutic drugs or research tools. In most cases, patents have been filed with the US patent office (USPTO), but for more significant discoveries, patents have been filed in additional patent jurisdictions (e.g. UK, Japan, Canada, China, and Hong Kong).

Equipment developed:

The near-single-cell resolution infrared laser-mediated heating system coupled with fluorescence microscope, developed for precise temperature control in living animals by Profs. Jiannan Qu and Zilong Wen, represents an outstanding output of this project. The two PIs have received a number of requests from the international developmental biology community on building such systems for their research.

6.8 Other education activities and/or training programmes developed:

TRS members have organized and participated in several workshops, symposia, and showcase events to promote their research interests and activities for public education, and to provide an environment/opportunity to facilitate international collaborations. In addition, some TRS members have also organized short projects and training programs for undergraduate students. Please refer to **Appendix 5** for details.

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

NA

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

Prof. Nancy Y. Ip and other core members set up a management committee (9 members) and an international advisory committee (4 members) to manage and advise the program. In addition, Prof. Ip organized the TRS meetings with the assistance of Profs. Zhenguo Wu (the deputy PC),

Mingjie Zhang (group leader for basic research) and Yung Hou Wong (group leader for translational research). Furthermore, she chaired all the meetings, updated team members on current news related to the program, and led and directed team discussions. Additionally, group leaders were also proactive in coordinating the activities of the program. Prof. Ip also often spoke to individual members to discuss their research progress. Moreover, since she has a vast network comprising local and international scientists in the stem cell and neuroscience fields, she has played an important role in connecting team members to prominent researchers in the field, as well as disseminating the latest developments in the field to the team members.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

TRS team members have been individually productive in securing research grants within their respective disciplines. Since 2012, a total of 11 research grants directly related to the TRS project have been awarded to team members. Please refer to **Appendix 6** for details.

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

The TRS team members have been regularly invited to present plenary or symposium lectures at international conferences and workshops both locally and overseas (total: 107). Team members have also been invited to organize international conferences (total: 24). Please refer to **Appendix 7** for details.

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Many of the TRS team members are in leadership positions on editorial boards of international refereed journals. Their work on TRS have received recognition by the scientific community and they have participated in professional organizations. Collectively, TRS team members serve as editorial board members in 53 scientific journals (*e.g. Progress in Neurobiology, Trends in Neuroscience, PNAS*) and member of 60 scientific and professional organizations. Please refer to **Appendix 8** for details.

7.4 Any documentary proof of the application of technologies arising directly from this project?

A number of patents or inventions have either been, or are in the process of being, secured. These include eight granted patents (CN103370338B; JP2016-526434; US9133258; US9150608; US9629830; US9629863; ZL201080055284.4; ZL201480039831.8) for four inventions, and seven pending patents (CA2918340; CN201180014384.7; EP20140826591; GB1218684.7; HK13104696.4; HK16108023.6; PCT/IB2018/053315). Details of the inventions and their corresponding patents/ patent applications are listed in Section 13.

7.5 Other awards and recognitions as a result of this project (please specify):

The TRS team is comprised of eminent researchers who are internationally recognized in their respective fields. The outstanding research and scholarship achievements of the team members have earned them numerous awards and recognitions. Selected examples are as follows:

- *Croucher Senior Research Fellowship Award*: Prof. Mingjie Zhang received the Senior

Research Fellowship from the Croucher Foundation in 2018. The Fellowships are awarded to distinguished scientists for publication and research work considered by their peers to be of critical importance to science and Hong Kong, thus meriting subvention.

- *Bronze Bauhinia Star*: Prof. Nancy Ip received the Bronze Bauhinia Star from the Hong Kong Government in 2017 for her strenuous efforts towards the advancement of science and education and dedicated commitment to the academia.
- *10 Science Stars of China by Nature*: Prof. Nancy Ip was selected by Nature magazine in 2016 as one of the 10 Science Stars of China. Ten leading scientists from China, who have made significant impacts in fields ranging from neuroscience and neutrinos to space science and structural biology, were highlighted.
- *Leader of the Year Award*: Prof. Mingjie Zhang was selected as the Leader of the Year by Sing Tao News Corporation in the education category in 2016 for his outstanding contributions to research and education.
- *Croucher Innovation Award*: Prof. Tom Cheung received the prestigious Croucher Innovation Award in 2015 for his distinguished scientific research achievements.
- *ACS OpenEye Outstanding Junior Faculty Award*: Prof. Xuhui Huang received the OpenEye Outstanding Junior Faculty Award in Computational Chemistry from the American Chemical Society in 2014.
- *Medal of Honour*: Prof. Yung Hou Wong received the Medal of Honour from the Hong Kong Government for his outstanding achievements in the biotechnological field in 2012.

8. Impacts

- 8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

In addition to the research accomplishments in stem cell and neuroscience described above, the achievements borne out of this TRS project contribute significantly to the social and economic development of Hong Kong. The project's major social impact is its contributions to enhance the understanding of, and develop effective therapeutic interventions for, neurological diseases such as Parkinson's and Alzheimer's diseases, clinical depression, and nerve injuries. This has significant implications, since currently there are few treatment options available. Hong Kong's population is rapidly aging as life expectancies are increasing. It is estimated that the elderly population (65 and over) will be ~1.8 million by 2029, around 21.5% of the projected total population. As the population ages, the number of people afflicted with neuro-related diseases and disorders will similarly increase because most of these are age-related conditions. At its present state, Hong Kong, much like other countries, is poorly-equipped to deal with an overburdened medical health-care system. The costs of treating and managing these diseases pose a heavy burden to patients, care-givers, and society. This TRS project has laid the foundation for applying neural stem cell-based approaches towards the development of effective treatments which will have a positive transformative impact on global health.

The project can also make a significant economic contribution to Hong Kong. Hong Kong is in the midst of developing its biopharmaceutical industry and its success in this venture is linked to the scientific research capabilities in the territory, the infrastructure, and access to a highly skilled work force. Our accomplishments from this TRS project have contributed in all three aspects. The scientific achievements from this project have unravelled complex molecular mechanisms and highlighted the high-level of basic research being conducted in neuroscience and stem cells in Hong Kong. This was achieved through a collaborative effort by different institutions and the

multidisciplinary team of esteemed scientists. Team members have also pioneered the use of several new, state of the art technologies to study neural stem cells and the brain. Alongside, the project has provided hands-on-training opportunities in research and development for students and research staff, to help develop skilled personnel required to support a biopharmaceutical industry in Hong Kong.

Translational research from this project has led to the discovery of neurogenic TCM compounds and small molecules, and conditions for axonal regeneration, which have potential therapeutic applications for treating various neurological diseases. Patent applications have been granted (or are in the process) for compounds identified from these studies (Section 6.7), and these are expected to give rise to new collaborations with drug-discovery industry to further their development (please refer to Section 6.5 for ongoing collaborations with industrial partners). These outcomes contribute to ongoing efforts to establish Hong Kong as a hub for biotechnology research, which in the long-term will help attract leading international biopharmaceutical companies to establish their R&D in Hong Kong, as well as encourage local entrepreneurs to invest in neural stem cell-related companies. Our demonstrated ability to secure patents and collaborate with biopharmaceutical companies will pave the way for future endeavours in technology transfer.

Stem cells have rapidly emerged as an important research area as they hold enormous potential for developing novel therapeutics for neurological diseases that are currently untreatable. Through the successful completion of this TRS project, we have established the essential foundations for conducting advanced basic and translational neural stem cell research in Hong Kong. Undoubtedly, this TRS project has effectively forged collaborations between local and international neuroscientists, as well as between local and international institutes (e.g. between HKUST and Karolinska Institutet; HKUST and Ecole Polytechnique Federale De Lausanne). Our activities have established Hong Kong scientists as major players in advanced neural stem cell research. We have amassed critical expertise, developed advanced capabilities, and established strategic collaborations with local and international institutes, all of which make significant inroads towards the long term development of Hong Kong.

8.2 Others (please specify):
NA

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

Yes. This TRS project was conceptualized to lay the essential groundwork for the development of neural stem cell-based regenerative treatments by combining approaches in basic and translational research and leveraging the complementary findings that arose therein. The success of this project is a result of synergistic interactions between the basic and translational research teams, and several new ideas have resulted from a series of brainstorming sessions undertaken by team members during the course of this project.

Having deciphered some of the complex details underlying proliferation and differentiation of neural stem cells, we conceived approaches to further harness these findings towards the development of therapeutic approaches to Alzheimer's disease (AD). Some of the technologies established during this project could be directly applied to further interrogate the pathological mechanism of AD. For example, we have proposed approaches using two-dimensional and three-dimensional neural cultures derived from human iPSCs obtained from AD patients to interrogate AD pathophysiology. Specifically, these patient-derived iPSCs present an attractive model for mimicking the genomic background in which sporadic AD develops. Furthermore, some

of the lead compounds identified during this TRS with neuroprotective capabilities could be further developed into candidate drugs and appropriate preclinical studies were proposed for this purpose.

In addition, specific new ideas that have evolved were implemented in studies within this project. Notable examples include:

- Based on the finding that cholecystokinin (CCK) is one of the key neurotransmitters that promote the neural plasticity and the formation of memory, CCK agonists and antagonists that could penetrate blood brain barrier (BBB) were designed by Prof. Jufang He's group. Their possible therapeutic effect on improving memory and learning in animal models are under investigation.
- Single-cell RNA-sequencing was established by Prof. Tom Cheung's group to uncover the transcriptional heterogeneity of neural stem cells, and for the activation status of muscle stem cells in their case. This technology was applied by Prof. Nancy Ip to delineate the lineage development of neural stem cell in adult hippocampus.

Finally, some new ideas that gave rise to testable hypotheses have resulted in the initiation of new projects (please refer to Section 9.2 for details).

9.2 Whether there are new projects evolved directly from this project?

Yes. In this TRS project, we explicated complex mechanisms governing the regulatory pathways that underlie neurogenesis and differentiation of stem cells, and identified and characterized a number of molecular players involved in these complex processes. Building upon the iPSC platform and the new findings that emerged from this TRS, we proposed a follow up TRS project entitled "A Stem Cell Approach to Dissect the Molecular Basis of Neurodegenerative Diseases". Funding for this TRS renewal has recently been approved. The successful completion of this subsequent project will bring us one step closer to developing novel therapies that can halt or reverse the devastating effects of AD

In addition, several of the investigators in this TRS have individually or collaboratively initiated new projects based on results obtained from this TRS. Research grants for this purpose have been successfully obtained from a variety of funding sources including the Research Grants Council and the Focused Innovations Scheme. A total of 14 separate proposals have been awarded, amounting to HK\$21.12M across 11 investigators. For further details please refer to Appendix 6.

9.3 Whether there are new collaborations developed directly from this project?

A total of 20 new collaborations, domestic and international, have been established as a direct result of this project. These collaborations make use of the collaborators' joint expertise in subject matter, technology, and resources to further develop upon some of the significant findings from this project, exploring both basic research and translational directions. Examples include:

- Profs. Mingjie Zhang (HKUST) and Quansheng Du (Medical College of Georgia, USA) have combined their respective expertise in structural biology and cell and molecular biology to investigate asymmetric cell division and polarity complexes critical during neurogenesis.
- Profs. Xuhui Huang (HKUST) and Nancy Ip (HKUST) have successfully employed computer-aided approaches in drug discovery. Prof. Huang utilized his expertise in computational biophysical chemistry and Prof. Nancy Ip contributed her expertise in bioassays and animal studies towards the discovery and characterization of novel drug targets.
- Prof. Jufang He (City U) collaborated with Profs. Duanqing Pei and Guangjin Pan (both from Guangzhou Institutes of Biomedicine and Health, CAS) to establish the co-culturing of primary rat cortical neurons and human iPSC-derived neurons.

Please refer to **Appendix 3** for a detailed list of all collaborations.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

HKUST recognizes the need to continually strengthen research excellence and build upon research successes. The University has provided a total matching funding of \$10.365M. Moreover, we have established a Center for Stem Cell Research (CSCR) at HKUST under this TRS project. The mission of CSCR is to position HKUST as a prominent center for stem cell R&D while stimulating interest from leading biopharmaceutical companies. The long term goals of this center are to position Hong Kong as a regional center for neural stem cell R&D and significantly boost Hong Kong's biopharmaceutical industry. The CSCR will expand on some of the work initiated from this TRS project, and has received seed funding of \$4.83M from HKUST.

In addition, the University's Technology Transfer Center supports the filing of US and UK patents upon a stringent review process. The cost of patent filing is covered by the University if the invention is deemed novel and has significant potential. A number of drug leads identified through the TRS project have already received approval for patent filing. Likewise, the R&D Corporation under HKUST is effective in IP asset management, developing collaborative industry-sponsored R&D projects, business networking and technology marketing, licensing, and incubation of start-up companies. Together, these two units within HKUST represent valuable resources for promoting R&D partnerships and technology transfer to assist in the economic development of Hong Kong. The patent cost is estimated to be \$4 million.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

No. of outputs arising directly from this research project	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
					Type	No.
	186	178	0	4	NA	NA

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

In 2012, a multi-disciplinary team of researchers from Hong Kong and abroad was assembled to investigate the basic mechanisms underlying neural stem cells and neurogenesis. Neural stem cells refer to the self-renewing, multipotent cells that give rise to brain cells during development. Understanding the complex mechanisms by which these cells originate, replicate, and function will further enable the development of stem-cell based therapies for many incurable neurological diseases.

The main objective of the project was to investigate the regulatory processes underlying neurogenesis, the process by which new brain cells are generated, as well as the processes that regulate the differentiation and maturation of newborn neurons, and their eventual integration into neural networks in the human brain. Alongside, a traditional Chinese medicine (TCM) –based approach was utilized to identify novel agents capable of promoting brain cell generation. These two approaches (basic research and translational) were used together to lay the groundwork for the future development of targeted neural stem cell based treatments.

The successful completion of this project has resulted in significant accomplishments. Findings from basic research have greatly enriched our understanding of the biology of neural stem cells. We have also developed advanced research capabilities and state of the art techniques such as single-cell RNA-sequencing, optogenetics, and deep brain imaging. We have uncovered the molecular mechanisms underlying the balance of neural stem cell division and differentiation – two essential processes for generating neurons in the brain. In translational research, we have discovered critical interactions between neural stem cell development and the pathology of anxiety and major depression. Moreover, by leveraging our TCM-based drug discovery expertise, we have identified novel agents from TCM with the ability to promote neurogenesis, neuronal differentiation or neuroprotection. Finally, we have established a platform to culture and study induced pluripotent stem cells (iPSCs) that can be further harnessed towards studying the pathological mechanisms of Alzheimer’s disease using patient-derived iPSC. These findings offer new hope for the development of effective clinical interventions for neurological diseases.

The project also provided essential training opportunities to local students and junior scientists, and both strengthened and extended collaborations among institutions in Hong Kong, the Mainland and abroad. The resulting scientific output from the project in the form of numerous publications in prestigious journals, patent applications, and presentations at international conferences, has highlighted Hong Kong’s excellent scientific research capabilities. The successful completion of this project further enhances Hong Kong’s reputation as an advanced research centre as well as contributes to the development of the local biotechnology industry.