Molecular Genetic Studies into the Development, Function, Pathology and Repair of Retinal Ganglion Cell Types

Habilitation Thesis

Tudor C. Badea, M.D., M.A., Ph.D.
Senior Researcher, Transilvania University

Abstract

I am presenting in this habilitation thesis a summary of my scientific investigations and my professional and academic progression since obtaining my PhD at Johns Hopkins University School of Medicine. I also outline some of my future projects as part of establishing a molecular genetics and neuroscience research program at Transilvania University.

My career progression (section B.III.1) is somewhat unusual. I obtained an M.D. from Iuliu Hatieganu University in Cluj, then pursued a pathology fellowship at University of Maryland. I then switched careers towards basic science, and obtained a Master of Arts in Biological sciences from Columbia University in New York, and Ph.D. in Biochemistry, Cell and Molecular Biology from Johns Hopkins. I then completed my postdoctoral fellowship in Molecular Biology and Genetics and Johns Hopkins School of Medicine. I then joined the USA National Institutes of Health in Bethesda M.D. as an investigator of the National Eye Institute and lead a group of undergraduates, Ph.D. and postdoctoral students for 11 years. I recently joined the Research and Development Institute at Transilvania University of Brasov in the Faculty of Medicine, where I am in the process of establishing a research group focused on molecular genetics and neuroscience.

My research resulted in 59 peer reviewed manuscripts, all published in WOS/ISI indexed journals, with a total of 3109 citations and an H-index of 27 (WOS, 28 December 2021). Of these, 48 papers were published since my graduation, including some in Nature, Cell, Neuron, PNAS and other high impact prestigious journals. Several manuscripts are under review/in press or deposited on BioRxiv. I am an editor for PLOSOne and Frontiers in Neuroscience, have reviewed manuscripts for more 20 WOS/ISI indexed journals, and served as grant reviewer for many funding bodies in Europe and the USA. I have mentored 23 students at Postdoctoral, PhD, M.Sc. and post-baccalaureate level, and served on many committees for recruitment, tenure or resource management at the NIH or elsewhere.

I am first briefly discussing the current state of the art in the field of neuronal cell type studies (section B.I), by reviewing anatomic, physiological, molecular and functional criteria for cell type definitions, and the lack of comprehensive surveys that combine all these criteria into a unitary concept. My major focus for the last 16 years have been Retinal Ganglion Cells (RGCs), the cells that carry visual information from the eye to the brain. Using RGCs as a test case, I illustrate how neuronal cell types can be defined and how their function within the system and their development can be studied. I then briefly describe the transcriptional mechanisms regulating RGC type formation, with a particular focus on Brn3/Pou4f transcription factor family, the major focus of my research in the last 15 years.

Section B.II lays out my contributions to these fields. Subchapter B.II.1.1 briefly describes my doctoral work on sparse random recombination as a tool to study neuronal cell types. This is necessary, as my postdoctoral work and the work of the laboratory I have headed at the NEI is based methodologically and conceptually on some of the tools and concepts I have pioneered during my PhD. Sections B.II.2 through B.II.8 describe my major contributions organized by topic and/or methodology. A significant aspect of my work has consisted in developing new genetic strategies for cell and gene manipulation (B.II.1), by generating conditional knock-in alleles, and Cre recombinase drivers. Through the intersection of these genetically modified mouse lines, specific cell types of interest can be labelled and/or manipulated. More recently we have also employed a second recombinase, Dre, in our genetic manipulations. Most of these genetic manipulations have helped me and my collaborators understand how transcription factors control RGC type specification. We have discovered cell autonomous mechanisms, transcriptional combinatorial codes, and interactions with neurotrophic signals (B.II.2). As a consequence, I have addressed potential molecular mechanisms for RGC type specification, by analyzing, in our group or through collaborations the transcriptional targets of Brn3 transcription factors in RGC type specification (B.II.2 and B.II.3). One particularly productive direction has been the study of ipRGCs (B.II.4), a specific class of RGCs that are intrinsically responsive to light by virtue of expressing the photopigment Opn4/Melanopsin. Using genetic manipulations employing some of the lines I have developed, I collaborated with colleagues at Hopkins, U. of Maryland and NIH and helped discover that ipRGCs can be subdivided in two subpopulations, responsible for circadian photo-entrainment and pupillary light reflex, respectively.

Similar transcriptional and signaling cascades regulate the development of multiple classes of projection sensory neurons (e.g. RGC, dorsal root ganglion and trigeminal ganglion somatosensory neurons, auditory and vestibular ganglion neurons). Thus, using our conditional knock-in alleles, we were able to achieve significant progress in the classification and anatomic description of these classes of neurons (B.II.5).

In order to analyze the consequences of our genetic manipulations on RGC electrophysiology and mouse visual function, we led a vigurous program of technique

development, by building ex vivo and in vivo functional analysis apparatus and software (B.II.6). We were therefore in position to make significant contribution to the characterization of a series of retinal developmental disorders, genetic defects, and disease models.

My initial background and education in biomedical research was centered on immunopathological mechanisms of autoimmune or degenerative disorders. More specifically, I began my research career in the USA as a Pathology fellow, studying the effects of complement on cell signaling and transcription in somatic cells, in University of Maryland, under the guidance of Horea Rus and Florin Niculescu. These studies, carried out before my PhD (B.II.7.1), resulted in the cloning and functional characterization of RGC-32 (more recently renamed to Rgcc), a gene involved in cell cycle regulation and phenotypic changes of a variety of somatic cells and immune system components. Subsequently, during my PhD, Postdoctoral and Independent Investigator years, I continued my collaboration with the Rus lab, and, together we generated a RGC-32 knockout mouse, which we employed to demonstrate RGC-32 involvement in several animal models for autoimmune disease and tissue fibrosis (Section B.II.7). I am particularly interested in the role of RGC-32 in pathology, since recent work from our group and others discovered the involvement of RGC-32 in epithelial-mesenchimal transition, and phenotypic programming of olygodendrocytes and astrocytes as a result of inflammatory cues. These changes occur in several neurological disorders including some that are important in visual pathology (e.g. Glaucoma, Multiple Sclerosis or Age-related Macular Degeneration).

Looking forward, I plan to develop a molecular genetics and neuroscience center at Transilvania University, progress to Senior Scientist I (Cercetator Stiintific I) or Professor, and develop a multidisciplinary research program involving students at all levels, from undergraduate to postdoctoral fellow. I hope to do this by interacting with a very diverse range of experts at Transilvania University, in other research centers in Romania and internationally. My research agenda will continue to be focused on RGCs, neuronal cell type development, and comparative studies between mouse and human systems. We will use our animal models to investigate pathogenetic mechanisms in visual system disorders, and explore vision restoration strategies based on bio-electronic interfaces, gene therapy or cell replacement/reprograming. This rich program will hopefully joined by PhD candidates from all connected fields, and will be strongly interdisciplinary.