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TEL AVIV UNIVERSITY אוניברסיטת תל אביב

הפקולטה למדעי החיים ע"ש ג'ורג' ס' וייז
המחלקה לחקר התא ולאימונולוגיה
פרופ' אורנה אלרואי-שטיין

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Research Update – December 2016

Orna Elroy-Stein's Lab, Tel Aviv University

2016 was an exciting year. Two additional PhD students (Mika Rotem and Shir Srour) joined the VWM research team in the lab (Dr. Andrea Atzmon, Reut Sharet and Melisa Herrero), making it a team of six dedicated people (4 grad students, a research assistant and myself as the team leader) working together aiming to discover the molecular pathways that goes awry as a result of eIF2B mutations. Alongside, we are engaged with the development of therapeutic commodities. During the last year we (i) learned more about the mitochondrial problems in eIF2B-mutant cells; (ii) Identified hyper-regulated proteins, the identity of which gave us clues about specific pathways that are skewed in eIF2B-mutants; and (iii) Finalized the drug screening project with a short list of 4 most-promising hits.

Mitochondrial Functions

Using part of the donations money we purchased a 'Seahorse XFe96 Analyzer' (http://www.agilent.com/en-us/products/cell-analysis-(seahorse)/seahorse-analyzers) which measures the oxygen consumption rate and extracellular acidification rate of live cells. These rates are key indicators of mitochondrial respiration and glycolysis; thus they provide a systems-level view of cellular metabolic function, under basal conditions and in response to various pharmacological treatments. Using this powerful new technology, Melisa and Reut studied in depth the mitochondrial deficits of eIF2B mutant cells which we already discovered last year. While Reut isolated primary fibroblasts (connective tissue cells) from the eIF2B-mutant mice, Melisa isolated glial cells (oligodendrocytes and astrocytes, both are known to be defective in VWM patients) from their brain and compared their performance to cells isolated from normal mice. It is now very clear to us that eIF2B-mutant cells suffer from compromised mitochondrial respiration, which results in energy deficits. Additional experiments revealed that compensatory molecular mechanisms are activated in eIF2B mutant cells to compensate for the energy deficits, but the compensation impact is not absolute. Moreover, we discovered that brain glial cells are much more sensitive to the effect of eIF2B mutation on mitochondrial performance and energy production, compared to fibroblasts. Since both astrocytes and oligodendrocytes require high energy level for their appropriate function, their hyper-sensitivity to the effect of eIF2B mutation

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explains the clinical symptoms of VWM, which are mostly confined to the brain. Future plans include multiple experiments to enhance the resolution of molecular pathways that are defective in eIF2B-mutant cells. To increase our chances for success, we recently used some of the donation money to purchase a fluorescent-activated cell sorter (FACS) (http://www.sonybiotechnology.com/sh800s) which enables the analysis of sub-populations of cells based on their differential features.

Drug screening

During the initial phase of the project, we developed a detection method based on single-cell imaging analyses using fluorescent detectors of mitochondria content, membrane potential, and level of reactive oxygen species (ROS). Using this tool, we screened the commercial DIVERSet™-EXP library which contains 50,000 drug-like compounds. Using a computational tool which takes into account the chemical and structural properties of each compound, this library was divided into 500 clusters, each represented by a single compound. The first screening round revealed 8 hits which together represent 437 molecules that were further screened in the second phase and yield 20 hits (final 0.04% hit rate). Hits that passed the toxicity tests were subjected to putative target identification using the Scifinder® database. Four putative targets (referring to specific molecular pathways) highly relevant to VWM biology were assigned to 7 hits. We then validated the mal-performance of these putative molecular targets in eIF2B mutant cells, which is an important discovery by itself. Excitingly, treating cells with the corresponding compounds hits increased mitochondria health, specifically in eIF2B-mutant cells. Importantly, one hit also reverses the hypersensitivity of eIF2B-mutant cells to pharmacologically-induced cellular stress and rescues them from the stress-induced cell death. The project not only illuminated specific cellular targets that were not known before as impaired in VWM cells, but also pointed at a specific potential drug, that improves eIF2B-mutant cellular phenotype and survival.

Future plans include multiple experiments to enhance the resolution of molecular pathways that are defective in eIF2B-mutant cells and further drug design-oriented experiments. With the addition of Shir and Mika, we hope for an exciting and successful 2017 !

We wish you Happy Holidays & Happy + Healthy New Year

Prof. Orna Elroy-Stein, PhD

on behalf of the VWM research team

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