Horizon 2020 MARIE SKŁODOWSKA-CURIE ACTIONS

Project Title: Genome editing for spatiotemporal analysis of centriolar satellite biogenesis and function in cellular stress responses

1. Summary of the context and overall objectives of the project

Cells contain specialized structures that perform a range of functions essential to maintaining the health of the cell. Defects in these structures or the functions they perform can manifest in a chronic disease state. Understanding how these structures contribute to the maintenance of a healthy state allows us to learn how diseases arise, and develop new strategies to prevent or treat them. One such structure is the centrosome, which resides in the centre of the cell. The centrosome contributes to a number of cellular processes, including cell division, and serves as the base of the primary cilium, an antenna-like structure that allows cells to respond to external stimuli. Around the centrosome lie a number of protein-containing granules, termed centriolar satellites. These are involved in the movement of proteins around the cell and are crucial for normal centrosome and cilium assembly and function. Currently little is known about the full role, composition, or regulation of these satellites, although mutations in a number of genes encoding satellite proteins are known to result in human disease, such as ciliopathies and neurological disorders.

The central scientific goal of this project is to expand our knowledge with regard to satellite function and contribution to maintaining a disease-free state. The realization of this goal will be achieved through a number of specific objectives, namely: the generation of a unique toolbox of reagents which we can use to study satellites; profiling of satellite protein contribution to normal

satellite function; visualization of satellite movement throughout the cell; and the definition of the contribution of satellites to cell stress responses. Furthering our understanding of the roles satellites play in the cell will greatly enhance our knowledge of how their dysregulation leads to disease. To achieve these objectives, we are coupling genome-editing and cutting-edge imaging techniques in novel approaches to explore this area of research. The Fellow is receiving extensive training in these techniques in the Pelletier Lab. Alongside these research skills, the Fellow is acquiring a range of complementary skills, allowing the fulfillment of the overarching objective of developing the Fellow's career to a position of professional maturity.

2. Work performed to June-30-2018 and main results achieved so far

Studying the consequences of removing a protein of interest from a cell can tell us a lot about the role that protein plays in the normal functioning of the cell. Thus, the project initiated with the generation of a number of cell lines lacking the satellite proteins we are interested in. This was achieved through employment of the CRISPR/Cas9 genome editing system. This technique leads to the disruption of the targeted genes, meaning the protein of interested is no longer expressed. To date, human cell lines deficient in 12 different proteins have been generated that are available for the fulfillment of the project's objectives. Alongside this, reagents for an alternative method of protein knockdown, siRNA, were optimized for use during the project. While these techniques were being developed by the Fellow a number of pharmacological inhibitors, that impact a range of cellular processes, were screened for their effect on satellites. Strikingly, it was observed that inhibition of the proteasome had a profound effect on the appearance of satellites within the cell. The proteasome is the complex that the cell uses to recycle the components that make-up unneeded or unwanted proteins. Therefore, the proteasome is crucial to regulating the concentration of proteins within the cell and preventing the toxic accumulation of damaged proteins. Inhibition of the proteasome leads to the collection of non-degradable proteins into a structure around the centrosome, called the aggresome. We found that all the satellite proteins examined became entangled within the aggresome following proteasome inhibition. When we looked at aggresome formation in the cell lines where we had used genome editing to remove satellite proteins, we discovered that this process was blocked. This means that satellites themselves are essential to the formation of the aggresome, which is an important observation for understanding how protein degradation is regulated. This allows us to build a model in which satellites contribute to the

movement of proteins to the aggresome, expanding on the previously reported requirement for dynein, microtubules and HDAC6 in this process. Furthermore, by closely examining the role of individual satellite proteins in aggresome formation, we have observed that a number of our proteins of interest are required earlier in the aggresome pathway, namely when proteins first start to aggregate within the cytoplasm. Knockdown of these proteins also prevents aggresome-like structures induced via alternative methods, such as the inhibition of protein translation and autophagy. These findings present a new model for the regulation of protein levels in cells and link centriolar satellites to a novel activity in cellular regulation.

3. Progress beyond the state of the art, expected results until the end of the project and potential impacts

Studies encompassing centriolar satellites generally reside in the centrosome/cilia field, while research focusing on aggresome formation belongs in the proteostasis field. This project has uncovered a novel link unifying these two fields, placing satellites in the regulation of protein degradation. Work continues to fully elucidate the specific mechanism via which specific satellite proteins contribute to this process. The accumulation of toxic protein aggregates is characteristic of neurodegenerative diseases, such as dementia, Parkinson's, Huntington's and Alzhiemer's. As aggresome formation represents a specialized cellular response to the failure of the proteasome machinery, current work is exploring whether these results have implications in neurodegenerative diseases, where the defective aggregation-prone proteins contain specific motifs that target them to aggresomes. Therefore, this project has the potential to profoundly increase our knowledge of how cells process these aggregates to prevent a disease state from developing. Increasing age is the biggest risk factor for the development of dementia, hence with an aging population dementia becomes more prevalent. Understanding how cells process protein aggregates, how this changes with age, and how these reach toxic levels will be fundamental to developing new therapies for the treatment, and potential prevention, of neurodegenerative diseases.