McGill University - Imperial College London Student Exchange
Training opportunity within the Fon Laboratory at the Montreal Neurological institute

Identifying novel substrates of parkin-mediated ubiquitination

Background: Over the past decade, the discovery of genes responsible for familial forms of Parkinson's disease (PD) has provided tremendous insight into the molecular and cell biological mechanisms involved. Mutations in the *Parkin* gene cause an autosomal recessive juvenile-onset form of PD that account for a large fraction of familial cases and probably contribute to sporadic PD as well. Importantly, several groups, including our own, have shown that the parkin protein functions as a key enzyme in the ubiquitin (Ub) system. Ubiquitin, a 76 amino acid protein, can be covalently attached to the ε-amino group of lysine (K) residues within target proteins. Ubiquitination requires the concerted activity of an E1 Ub-activating enzyme, an E2 Ub-conjugating enzyme and an E3 Ub-ligase. E3 Ub-ligases, such as parkin, form the largest family of enzymes and are involved in substrate recognition, thereby conferring specificity to ubiquitination. Despite the large amount of work carried out to understand parkin function, we still do not know the identity of most parkin substrates.

Opportunity: The aim of this proposal is to identify substrates of parkin-mediated ubiquitination in brain. This has been difficult to date because reagents (i.e anti-Ub antibodies) to purify and analyze ubiquitinated proteins from tissue have not been efficient. We plan on overcoming this limitation by crossing transgenic mice expressing HA-tagged Ub with parkin knockout mice in our lab. This will allow us to use anti-HA affinity chromatography to efficiently purify HA-Ub-protein conjugates from wild-type and parkin knockout brain samples. The samples will then be analyzed by mass spectrometry. We predict that *bone fide* endogenous parkin substrate will be recovered in samples from wild-type but not parkin KO brains. Newly identified substrates will be validated in brain and cultured neurons from parkin KO mice. As there is still much we do not know about the basic mechanisms of parkin function and its substrates, we predict this work will have major implication for our understanding of PD pathogenesis.

Role of visiting student: Under the guidance of a postdoctoral fellow, the student will be responsible for mouse brain tissue preparation and anti-HA affinity chromatography experiments. The student will optimize experimental conditions using SDS-PAGE followed by Coomassie staining prior to mass spectrometry. The student will also be intimately involved in the analysis and validation of the mass spectrometry data obtained.

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