2016Joint Usage and Research Report

Title of Research Project		Regulation of Akt by a novel ubiquitin E3 Ligase, RNF144A
Applicant	Institution	King's College London
	Job title and name	Principal Investigator/Sr. Lecturer
Visiting researcher	Name	Dr. Susan John
Purpose of the Research Project (approx. 250 words)		AKT (or protein kinase B, PKB), is a serine/threonine protein kinase that is activated downstream of the lipid kinase Phosphoinositide 3-kinase (PI3K) following growth factor, cytokine or antigen receptor stimulation. AKT is a critical signalling molecule that regulates and coordinates diverse cellular functions such as, cell size, proliferation, survival, translation, metabolism, and motility. The critical function of AKT in maintaining normal cellular homeostasis is underscored by human diseases such as cancers, metabolic disorders, Down's Syndrome and Type-1 Diabetes, where AKT function is either hyperactivated or inactivated. In light of its vital role in controlling cellular homeostasis, AKT function is tightly controlled under normal physiological conditions. One important regulatory mechanism by which AKT activity is regulated is ubiquitylation. To date a few different E3-Ubiquitin ligases have been identified which can ubiquitylate AKT or the active phosphorylated-AKT resulting in either proteasomal degradation or promote nuclear localization. We have recently, identified a novel cytokine induced E3-Ub ligase, RNF144A, whose activity inhibits the AKT/mTOR pathway. In this collaborative study we wish to evaluate if there is a physical and functional interaction between AKT and RNF144A and whether RNF144A regulates Akt mediated autophagy in lysosomes.
Development of the Research Project and Results (approx.. 850 words)		The physical interaction between AKT (plasmid kindly provided by Prof.Noguchi) and RNF144A was investigated. 293T cells were transfected with FLAG-AKT and

	Myc-RNF144A and total lysates prepared and immunoprecipitated with a FLAG antibody to immunoprecipitate AKT. The immunoprecipitates were subjected to SDS-PAGE and western blot analysis performed with an anti-myc antibody to detect any co-precipitating RNF144A. The results showed specific co-precipitation of RNF144A with AKT, suggesting that the two proteins can interact together in a complex. Given that there is a physical interaction between the two proteins, we next investigated whether RNF144A can ubiquitinate AKT. 293T cells were transfected, with myc-RNF144A and FLAG-AKT, or either plasmid alone or empty vector alone. Immunoprecipitation analysis was performed using an anti- FLAG antibody to precipitate AKT, followed by SDS-PAGE and western blot analysis to detect ubiquitination using an anti-ubiquitin antibody (VU-1). The ubiquitin antibody detected polyubiquitination of AKT when RNF144A was co-expressed with it, but not in either control samples. Furthermore, the addition of a proteasomal inhibitor MG132 to the transfected cells showed increased AKT protein levels when RNF144A and AKT were co-expressed. This result suggested that RNF144A could induce polyubiquitination leading to proteasomal degradation of AKT. Thus we have made the novel observation that RNF144A is an E3-Ubiquitin ligase that potentially regulates AKT levels. We will next, confirm the nature of the ubiquitin linkage on AKT by using lysine mutant constructs of ubiquitin. Based on these results, experiments have been planned during Dr. John's visit to Prof. Noguchi's laboratory at Hokkaido University to investigate the functional impact of the regulation of AKT by RNF144A in autophagy. Prof. Noguchi's laboratory has recently identified a role for AKT in autophagy mediated by lysosomes. RNF144A knockout cells show a gene expression profile that is indicative of dysregulation of the autophagic process and increased AKT levels, and therefore we hypothesize that the RNF144A mediated degradation of AKT will be important for AKT function in lysosomes. In the future work planned, Prof. Noguchi's laboratory will assess the co-localization of

	RNF144A and AKT in lysosomes, and the function of AKT in lysosme mediated autophagy in RNF144A knockout cells using lentiviral mediated delivery of shRNA to knockdown RNF144A (lentiviral vector provided by Dr. S.John). Thus, our studies currently identify AKT to be a novel substrate for RNF144A with potential important functional implications for cellular processes regulated by AKT, such as autophagy and metabolism.
Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】 Work still in progress. Too early to publish yet.
	【Journals】