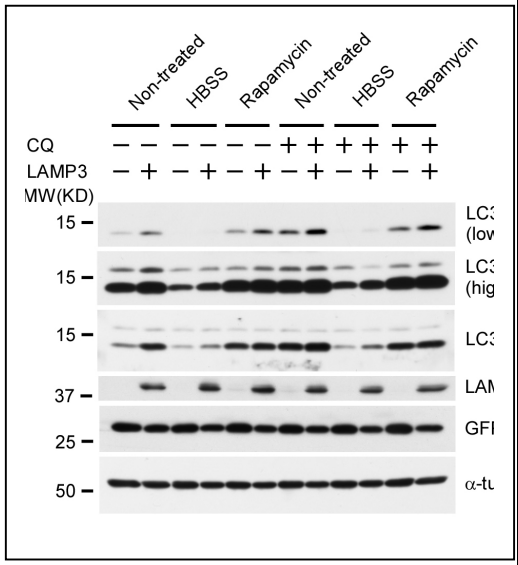
2015 Joint Usage and Research Report

Title of Research Project		
Applicant	Institution	National Institute of Dental and Craniofacial Research, Molecular Physiology and Therapeutics Branch
	Job title and name	Senior Investigator and Deputy Branch Chief
Visiting researcher	Name	John Chiorini
Purpose of the Research Project (approx. 250 words)		A major problem facing medicine today is defining the mechanism associated with the onset and symptoms of disease and developing treatment for them. With the reported increase in the prevalence and incidence of autoimmune diseases this group of diseases represents a major health concern. Sjögren's syndrome is the second most common autoimmune disease and is estimated to affect over 35 million individuals worldwide. This disease is characterized by a poor quality of life due to secretory epithelial cell dysfunction with dry mouth and dry eyes, lymphocytic infiltration within the affected salivary gland tissue, development of autoantibodies, and a 30 fold increased risk of developing lymphoma. Currently, no effective treatment exists for Sjögren's syndrome and our understanding of the physiological mechanism associated with the xerostomia is not clear. Analysis of the transcriptome can provide a global perspective of the physiologic state of the cell and identify specific as well as broad changes within the cell. Our lab has utilized this approach to investigate central questions related to Sjögren's syndrome such as the gene expression changes associated with the loss of gland activity. Through this analysis we have recently discovered many cellular changes in the gland associate with Akt signaling and autophagy. Correspondingly Dr. Noguchi' s laboratory has discovered by means of yeast two hybrid screening using full-length Akt as a bait, a connection with critical proteins in the autophagy pathway, specifically Phafin2. The collaborative work with Dr. Noguchi's laboratory should clarify the role of Akt and the induction of autophagy in the initiation and progression of Sjogren's syndrome. In this research proposal, we will use recombinant DNA experiments as well as animal studies.

Development of the Research Project and Results (approx.. 850 words)	Autophagy is originally characterized as a conserved process by which cells recycle cytoplasm and defective organelles (i.e. mitochondria) in diverged organisms from yeast to mammals. The process is associated with degradation and recycling during stress situations such as nutrient starvation (Klionsky, 2005; Levine et al., 2011; Mizushima et al., 2008). Recent study demonstrated that autophagy plays an important role in vivo physiology, so that malfunctioning of autophagy underlies various pathological condition including autoimmune diseases such as primary Sjögren syndrome (pSS) (Klionsky, 2005; Levine et al., 2011; Mizushima et al., 2008). We have previously shown that phafin2 associated Akt can translocalize to the lysosome, which is critical for induction of autophagy(Matsuda-Lennikov et al., 2014). This observation strongly supports that Akt can play an important role in the induction of autophagy. In an effort to identify the molecules underlie the pathogenesis of Sjögren syndrome, we conducted DNA microarray analysis to identify differentially expressed genes in Sjögren syndrome patients. We found that LAMP3, (lysosomal associated protein 3, formerly called Dendric Cell LAMP) was enriched over 5 fold in pSS. In our effort to work with Dr. Noguchi' s laboratory using lenti-virus expression systems, we overexpressed LAMP3 in HT1080 cells, and measured the level of autophagic induction by LC3 western blot. We found that LAMP3 overexpression clearly induce autophagy. Moreover, LAMP3 physically associated with Akt in co-immunoprecipitation assay in HEK293 cells. These observation supported the potential role of LAMP3 in the pathogenesis of pSS. **Method:** HT1080 cells were transduced by Lentiviral HA-LAMP3 transduction (pCSII-CMV-IRES2-Venus). Cells were treated with HBSS or 

	Rapamycin to induce autophagy and harvested and subjected to immunoblot with LC3 antibody. In our contining international collaboration with Dr. Noguchi, we will further clarify the functional role of LAMP3-Akt interaction as an underlying mechanisms of pSS. Given the high frequency of clinical association of lymphoma in pSS, functional interaction of the Akt-LAMP3, which is overexpressed in pSS patients, may represent a critical molecular mechanisms associated with this complication. Klionsky, D.J. (2005). The molecular machinery of autophagy: unanswered questions. J Cell Sci *118*, 7-18. Levine, B., Mizushima, N., and Virgin, H.W. (2011). Autophagy in immunity and inflammation. Nature *469*, 323-335. Matsuda-Lennikov, M., Suizu, F., Hirata, N., Hashimoto, M., Kimura, K., Nagamine, T., Fujioka, Y., Ohba, Y., Iwanaga, T., and Noguchi, M. (2014). Lysosomal interaction of akt with phafin2: a critical step in the induction of autophagy. PloS one *9*, e79795. Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008). Autophagy fights disease through cellular self-digestion. Nature *451*, 1069-1075.
Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】
	【Journals】