2019Joint Usage and Research Report

Title of Research Project		The ADP-Ribosyltransferase, TIPARP, and mono-ADP-ribosylation are critical mediators of AHR-dependent regulation of inflammation and immune responses
Applicant	Institution	Institute for Genetic Medicine
	Job title and name	Professor Akinori Takaoka
Visiting researcher	Name	Professor Jason Matthews University of Oslo
Purpose of the Research Project (approx. 250 words)		The Aryl hydrocarbon receptor (AHR) is a transcription factor that regulates the expression of many genes at the interfaces among environmental clues, metabolic alterations, and the immune system The AHR has historically been studied due to its ability to mediate the toxic responses of environmental pollutants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD/dioxin). It is now recognized as an essential gatekeeper integrating signals from the diet and endogenous metabolism to modulate immune cell homeostasis, reactive oxygen species, immunosuppression and tumorigenesis. The AHR regulates T cell differentiation, immune tolerance and innate immunity. Through this collaboration, we reported that TIPARP is the key regulator of AHR-dependent innate immune suppression and found that it specifically ADP-ribosylates TBK1 reducing its ability to activate type I interferon responses. (Yamada et al. Nature Immunology 2016). However, we still know very little about TIPARP's role in these processes, how it contributes to adaptive immunity, how the TIPARP-AHR axis is regulated during the innate/adaptive immune responses, and the role of TIPARP in AHR-dependent B or T cell function. Moreover, the inhibition of TIPARP may serve as potential therapeutic strategy to boost cancer immunotherapy by reversing tumour associated immunosuppression through increases in type I interferon signaling. Our proposed project will include comprehensive studies of Tiparp knockout mice, primary and immortalize cell line models, as well as genomic and proteomic methods to characterize the importance of TIPARP in the immune system and cancer immunotherapy.

Development of the Research Project and Results (approx.. 850 words)	During the past year my lab gained expertise in characterizing type I interferon response after treatment with synthetic pathogen-associated molecular patterns (PAMPs) like 3pRNA and cGAMP), through the sharing of protocols and material from Prof. Takaoka's group. For these studies we have focused on mouse embyronic fibroblasts (MEFs) from our Tiparp-/- mice and from our Tiparp catalytic mutant mice TiparpH532A. We found that exposure to PAMPs, as well as LPS dramatically increases the expression of several proinflammatory cytokines in addition to type I interferons. Our preliminary data suggest that TIPARP targets multiple components of the nuclear factor kappa-light-chain-enhancer of activated B (NFkB) signaling pathway and that NFkB activation regulates TIPARP expression independently of AHR. This suggests that TIPARP is part of a novel negative feedback loop regulated NFkB signaling, which could have important implications in inflammatory diseases. We are working to identify the mono-ADP-ribosylated peptides in NFkB. Another research focus of this work will be to investigate the role of TIPARP in T and B cell differentiation of function. Once isolated, the cells will be differentiation and the presence of absence of AHR ligands to determine the impact of TIPARP and AHR and T and B cell differentiation and function. These studies are ongoing. Together with a collaborator in the USA, we have identified a selective TIPARP inhibitor that we are currently testing in our various MEF lines. In preliminary studies, the new inhibitor phenocopied the effect of TIPARP loss and genetic catalytic inhibition. We are now poised to begin in vivo studies to determine if pharmacological inhibition of TIPARP could be a beneficial anti-viral therapy, but also cancer immunotherapy because of its increase in type I interferon signaling. Finally, we are creating a panel of CRISPR/Cas9 generated TIPARP knockout cell lines to determine the potential role the TIPARP in cancer. We our focusing on breast, liver, lung and pancreatic cancer cells. It has proven to be more difficult to create and isolate TIPARP ko cell lines, but we have created two breast cancer lines, to date. We have altered our strategy to improve knockout cell generation. We have also finally generated a mouse monoclonal antibody that recognizes TIPARP. This antibody has taken us almost 4 years to produce and as far superior to all of the current

	commercially available ones. Our unique MEF cell lines and animal models provide excellent material to test the specificity and quality of the antibody. This is a major advancement in TIPARP research, since the majority of studies have been done using overexpressed proteins, transient transfection or stable cell lines. We still know very little about endogenous TIPARP signaling, its cellular location, cellular targets and how it is regulated at the protein level. Ongoing studies reveal that TIPARP inhibition either pharmacologically or genetically results in increased stabilization of TIPARP protein. This supports our previous view that TIPARP is rapidly degraded and that it acts to regulate protein levels via ADP-ribosylation. The antibody cross reacts with human and mouse TIPARP and works in western blots, immunoprecipitations and immunohistochemistry, albeit not perfectly. We are starting a series of experiments to probe the role of endogenous TIPARP in AHR and type I interferon signaling. However, before we can expand our studies we need to generate more anti-TIPARP antibody from our hybridoma cells. The antibody was first isolated in early December of 2019. Our mouse models will be used to evaluate the role of TIPARP in cancer immunotherapy in combination with immune check point inhibitors to boost therapeutic responses. Inhibiting immune checkpoint proteins, such as programmed cell death protein 1 (PD1), and its ligand, programmed death-ligand 1 (PDL1), have shown promise in the treatment for multiple cancers by blocking immunosuppression. PD1 expressed on T cells counters positive signals through T cell receptors by engaging its ligand PDL1. During infections and under normal physiological conditions, the PD1/PDL1 axis is an essence negative regulator of T cells. Blocking the PD1 pathway during cancer, improves T cell function and reduces tumour burden. PD1 pathway inhibitors promoted durable anti-tumour immune responses in several trials, which led to the approval of the monoclonal antibodies raised against PD1 (nivolumab and pembrolizumab), or against PDL1 (atezolizumab, and avelumab) for therapeutic use in various cancers, including melanoma, non-small-cell lung cancer, breast and hepatocellular carcinoma. However, many patients do not respond and 1/3 of those that do, experience relapse. Thus, efforts to increase the effectiveness of

	immunotherapy are urgently needed. We believe the TIPARP is a promising target that should be considered. In the upcoming year, if our joint research application is successful, we will explore the role of TIPARP inhibition to boost the effectiveness of cancer immunotherapy. We feel that this ***novel***, research strategy to characterize the role of TIPARP in type I interferon response offers great potential for both anti-viral but also anti-cancer therapy. Our complementary expertise and access to unique animal and cell line models for studying TIPARP activity, give us an advantage over other labs pursuing this line of research. Continued support from the joint research program will ensure the success of this research.
Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】 Local department seminars at the University of Oslo.
	【Journals】 Yamada et al Nature Immunol 2016 Jun;17(6):687-94. doi: 10.1038/ni.3422. Epub 2016 Apr 18.