2022 Joint Usage and Research Report

<table>
<tr><td colspan="2">Title of Research Project</td><td colspan="3">Targeting PARP7-AHR axis to Improve Cancer Immunotherapy</td></tr>
<tr><td rowspan="2">Applicant</td><td>Institution</td><td>University of Oslo</td><td>Under40<br>put a ○</td><td>Under35<br>put a ○</td></tr>
<tr><td>Job title and Name</td><td>Professor Jason Matthews</td><td></td><td></td></tr>
<tr><td rowspan="4">Research collaborators<br>(Please add lines as appropriate)</td><td>Institution</td><td></td><td></td><td></td></tr>
<tr><td>Job title and Name</td><td></td><td></td><td></td></tr>
<tr><td>Institution</td><td></td><td></td><td></td></tr>
<tr><td>Job title and Name</td><td></td><td></td><td></td></tr>
<tr><td colspan="2">Host researcher at IGM</td><td colspan="3">Professor Akinori Takaoka</td></tr>
<tr><td colspan="2">Purpose of the Research Project (approx. 250 words)</td><td colspan="3">Poly-ADP-ribose polymerase 7 (PARP7) is upregulated in response to cellular stress, such as genomic instability, and the aryl hydrocarbon receptor (AHR), a ligand activated transcription factor. Using a variety of in vitro models and whole-body and tissue specific Parp7-/- mice, we reported that Parp7-/- are have enhanced AHR and IFN responses. PARP7 negatively regulates IFN signaling, which in the context of cancer allows tumor cells to “hide” from immunosurveillance. Since these actions of PARP7 depend on its catalytic activity, PARP7 loss or its inhibition in tumor cells should release the “brake” that cancer cells use to evade the immune system. Recently, small molecule inhibition of PARP7 was shown to restore IFN signaling and in essence release the brake that cancer cells use to evade the immune system. However, PARP7 also negatively regulates AHR, and its loss increases AHR signaling. Although AHR is best known for mediating dioxin toxicity, AHR drives pro-survival processes via kynurenine-dependent mechanisms to increase tumor growth and promote an immunosuppressive tumor microenvironment. When co-expressed with PARP7, AHR activation would be expected to counteract the anti-tumor effects of PARP7 inhibition. This suggests that inhibiting both PARP7 and AHR may offer therapeutic benefit for patients with tumors that express both proteins.<br>We hypothesize that loss or inhibition of PARP7 will reduce</td></tr>
</table>

| | |
|---|---|
| | tumor growth by increasing anti-tumor immune responses; however, for tumors that express AHR, PARP7 inhibition will increase tumor growth. Therefore, AHR inhibition will improve the therapeutic benefit of PARP7 inhibition. |
| Development of the Research Project and Results<br>(approx.. 850 words)<br>*Enter the number of web meetings. | Over the course of the past year, we have exchanged emails and ideas related to the research supported by the Joint Research Program. A formal web-based meeting was not organized but will be done in the future until other alternatives are possible. Due to uncertainty of travel restrictions, a physical visit did not occur. A physical visit to the host lab is planned for 2024.<br><br>**Results:**<br>**WP1: Determine the effect of inhibition/loss of PARP7 on tumor cell growth.** Here we proposed to test the intrinsic role of PARP7 in human and murine tumor cells. We will use the following human cell lines, MDA-MB-231 and MDA-MB-468 cells, and murine cell lines, EO771, and PyMT cells that were derived from murine mammary tumors.<br><br>***Task 1.1. Generation of PARP7 knockout cancer cell lines.*** CRISPR/Cas9 targeted gene editing will be used to generate PARP7 knock-out ($PARP7^{ko}$) and $AHR^{ko}$ human breast cancer cell lines, MCF7, MDAMB231, MDAMB468) and mouse breast cancer cell lines: EO771, PyMT). Not EO771 cells, were found to be AHR negative, so generating $AHR^{KO}$ cells was not necessary. As expected, $PARP7^{ko}$ increased type I IFN and AHR signaling. $AHR^{ko}$ differentially affected type I IFN, which varied among cell lines. No AHR signaling was observed in $AHR^{ko}$ cells.<br><br>***Task 1.2. Investigate and biochemical characterize novel PARP7 inhibitors.*** We tested 3 different PARP7 inhibitors all of which resulted in increased type I IFN and AHR signaling.<br>WP 2: Determine the effects of PARP7 immune cell-dependent tumour cell killing. This WP included 2 task: Task 2.1. Co-culture studies with human cans and cytotoxic T lymphocytes; Task 2.2. Co-culture of cancer cells with CD8+ T lymphocytes of macrophages from PARP7+/+ or PARP7-/- mice. We made little progress on these tasks during the past year. |

| | |
|---|---|
| | ***WP3. Determine the impact of inhibition/loss of PARP7 on mammary tumor growth in vivo.*** In this WP, we used Parp7$^{H532A}$ mice in which a single point mutation was introduced into the PARP7 catalytic domain, resulting in a PARP7 protein devoid of catalytic activity. We injected Parp7WT and Parp7$^{ko}$ EO771 cells in syngeneic tumor models using our Parp7$^{+/+}$ or Parp7$^{H532A}$ mice as hosts. These studies will determine differences between loss of PARP7 activity in tumor cells but not the host, versus loss of PARP7 activity in the host but not in tumor cells on solid tumor growth. In xenograft studies, PARP7 loss had no effect on tumor growth. In contrast, we observed that injection of wildtype cells into catalytically-dead *Parp7$^{H532A}$* mice resulted in smaller tumors compared with cells injected into *Parp7$^{+/+}$* mice. *Parp7$^{H532A}$* mice injected with Parp7$^{KO}$ cells failed to develop tumors after 30 days and those that developed regressed. Similar studies are planned for PyMT PARP7$^{ko}$ cells that express AHR to test the impact of PARP7 loss on tumor growth in cancer cells that express AHR. These studies are ongoing.<br><br>In task 3.2, we will determine the effect of loss of PARP7 catalytic on spontaneous mammary tumor growth by using the mammary specific polyomavirus middle T antigen (MMTV-PyMT) to generate a MMTVPyMT; Parp7$^{H532A}$ line. The MMTVPyMT mice have been purchased on the experiments should be completed by in early 2024.<br><br>Overall, progress has been good on the project, except for WP2, and we look forward to a continued strong research collaboration in the upcoming years. |
| Publication<br>*Enter the information of conference or journal (vol. page. Year.) where the above work was presented. | 【Conference, symposium, workshop etc.】<br>PARP7 2022, Cold Spring Harbor March 2022. |
| | 【Journals】<br>Some of this work will be submitted to for publication in the late spring of 2023. |