2018 Joint Usage and Research Report

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<tr><td colspan="2">Title of Research Project</td><td>The protective role of ANT2 in chemotherapy-induced liver damage and its therapeutic potential in cancer chemotherapy (2nd year)</td></tr>
<tr><td rowspan="2">Applicant</td><td>Institution</td><td>Harbin Institute of Technology, China</td></tr>
<tr><td>Job title and name</td><td>Postdoc
Kai Li</td></tr>
<tr><td>Visiting researcher</td><td>Name</td><td>Kai Li</td></tr>
<tr><td colspan="2">Purpose of the Research Project (approx. 250 words)</td><td>Chemotherapeutic agents are metabolized and eliminated mainly in the liver, and most are capable of causing liver damage including elevation of inflammation. Our study has also shown that mice were injected intraperitoneally (i.p.) with doxorubicin can significantly induce the expression of inflammatory cytokines (IL-6, IL-1β, TNF-α) in liver. Reduction of the inflammation may protect the liver from chemotherapy-induced damage and improve the therapeutic effect.
ANT2 (Adenine Nucleotide Translocase-2) is a mitochondrial protein that facilitates the exchange of ADP and ATP across the mitochondrial membrane. Previous studies have indicated that knockdown of ANT2 sensitized cancer cells to anticancer drugs. However, the role of ANT2 in chemotherapy-induced liver damage and its therapeutic potential in cancer chemotherapy are still poorly understood.
Our ongoing project has indicated that the expression of ANT2 and several cytokines (IFN-β, IL-6, TNF-α and IL-1β) are induced by chemotherapy drugs (Doxorubicin, Cisplatin and Etoposide), and suppression of ANT2 results in an elevated expression of cytokines upon these drugs treatment. Moreover, we found that the production of cytokines is dependent on the activation of cGAS-STING signaling pathway via sensing of tumor cell-derived mtDNA, and ANT2 has an inhibitory function on cGAS-STING signaling by repressing the cGAS reaction product-cGAMP binding to STING due to its competitive cGAMP binding activity.
Therefore, we hypothesize that ANT2 may function as a protective factor in drugs-induced liver damage by inhibition</td></tr>
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	of STING-mediated inflammation in liver, and this research project wants to clarify the protective role of ANT2 on chemotherapeutic drugs-induced liver damage and elucidate its therapeutic potential in cancer chemotherapy by using *in vivo* mouse model.
Development of the Research Project and Results (approx.. 850 words)	**Research Project** Due to the whole-body knockout of Ant2 is lethal, and liver specific knockout of Ant2 in mouse has been shown a normally growth as wild-type mouse and did not show any dysfunction of mitochondrial in liver, therefore, we will use Cre-mediated liver-specific deletion of Ant2 mice (liver-ANT2 cKO) in this study to evaluate the protective role of Ant2 in chemotherapeutic drugs-induced liver damage. To understand weather this protective role of Ant2 is caused by inhibition of STING-mediated cytokines production, we also will use liver-STING cKO and liver-ANT2/STING double cKO mice in this study. In detail, in this research, we will use four types of mice (WT, liver-ANT2 cKO, -STING cKO, -ANT2/STING double cKO mice). After the mice injected intraperitoneally (i.p.) with different dose of doxorubicin for 24, 48 and 72 h, we will analysis: (1) liver damage by detection cell apoptosis using TUNEL assay and western-blotting to detect apoptosis biomarkers caspase 3 and PARP; (2) liver damage by detection AST and ALT level in serum using ELISA; (3) Liver damage by using mice liver section HE stain; (4) Inflammatory cytokines production by qRT-PCR detection the mRNA level in liver and ELISA detection protein level in serum; (5) Mice survival after doxorubicin injection; (6) Macrophage infiltration in liver; (7) ANT2 induction in mice liver by using qRT-PCR and western-blotting; (8) Activation states of STING signaling pathway by using western-blotting to detect pIRF3 and STING dimerization. **Progress and results** 1. By using ANT2 liver KO-mice, we found that (1) ANT2 liver KO-mice has a normal growth compare with WT mice; (2)

	after intraperitoneally(i.p.) injection with doxorubicin, the ANT2 liver KO mice shown a decreased survival; (3)ANT2 liver KO mice had higher inflammatory cytokines (IL-6, TNF-α and IL-1β) and aminotransferase (AST, ALT) production after i.p. injection with doxorubicin; (4) HE stain and TUNEL stain detection also found that the liver damage was more serious than WT mice after doxorubicin treatment; (5) ANT2 protein expression was induced by doxorubicin treatment in WT mice liver. All the above in vivo results we observed suggested that ANT2 can function as a protective factor in drugs-induced liver damage. 2. A high impact journal manuscript "ANT2 functions as a negative regulator in the DNA sensing pathway via STING and protects hepatocytes from chemotherapy-driven liver damage" Kai Li, Seiichi Sato, Kozo Ishikawa, Chikashi Obuse, Naohiro Terada, Akinori Takaoka et al., 2019 is in preparation. **Remaining Work and Expected Results** 1. We still do not obtain STING and ANT2 double liver KO-mice after crossing the ANT2 liver KO-mice and STING whole body KO-mice, so we still do not understand the protective function of ANT2 in drugs-induced liver damage in vivo is via inhibition of STING signaling pathway or not, but from our cellular experiments, we expected that this protective role is via STING signaling pathway. 2. The protein level of ANT2 in mice liver was induced after intraperitoneally(i.p.) injection with doxorubicin, however, we did not identify the different phosphorylation site(s) in ANT2 by using LC-MS/MS analysis.

Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】
	【Journals】