2017Joint Usage and Research Report

Title of Research Project		Elucidation of the chemoresistance mechanism of ANT2 and its therapeutic potential in cancer chemotherapy
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Purpose of the Research Project (approx. 250 words)		It has been reported that IFNs play a multifaceted role in the induction of antitumor immunity. In addition to their direct cytotoxic effects on tumor, targeting low doses of type I IFNs to the tumor microenvironment also promotes the maturation and antigen presentation of DCs and boosts endogenous NK or $CD8^+$ T cell-mediated antitumor immune responses. Endoplasmic-reticulum (ER)-membrane protein STING is thought to function as an adaptor protein, which links upstream DNA sensors to downstream IRF-3 and NF-κB pathway activation. Recent studies have demonstrated that STING plays a key role in antitumor immune response. After transplantation of immunogenic tumors in STING-deficient mice, the tumors grow more rapidly than transplantation in wild-type mice or TRIF-deficient mice. Spontaneous $CD8^+$ T cell priming against tumors was also defective in mice lacking STING, but not in those lacking TLRs, MyD88 or MAVS, suggesting cytosolic DNA sensing pathway is involved in controlling tumor growth. Results from our ongoing project show that ANT2 has an inhibitory function on cGAS-STING signaling pathway. Therefore, we hypothesize that ANT2 may negatively regulate STING-mediated antitumor response during cancer chemotherapy, targeting ANT2 to boost antitumor activity of STING will hopefully provide a promising therapeutic strategy against tumor progression. In conclusion, the main purposes of the collaboration are: 1. Elucidating the role ANT2 and STING in chemotherapy-induced cell death. 2. Investigating the detail mechanism of ANT2 negatively regulating SING pathway. 3. Understanding the role of ANT2 in chemoresistance and its relationship with innate immune signaling pathway.
Development of the Research Project and Results		**Research Project** The role of STING and its role in innate immune response is

(approx.. 850 words)	well characterized. And recent works also reported that activation of STING by tumor-derived DNA for IFNs production and DC-mediated cross-priming is critical for generating adaptive antitumor immunity. In additional, activation of STING pathway results in production of IFNs for inducing ISGs to prompt cell death, it also can cause cell death via IFNs-independent manner, which is through STING-mediated IRF-3 interaction with Bcl-2-associated X protein (Bax) on mitochondria to activate the mitochondrial apoptosis pathway dependent on caspases 9 and 3. Therefore, triggering of STING signaling can directly induce cancer cell death and may provide a new cancer therapy option. However, it has been demonstrated that apoptotic caspases suppress the activation of STING pathway. Hence, a feedback regulation between STING and apoptosis signaling pathway may exist; trying to release this inhibitory effect of apoptotic caspases on STING may enhance the anticancer activity of STING. 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 the expression of ANT2 is upregulated in several types of cancer, knockdown of ANT2 showed decreased cancer cell migration/invasion and enhanced sensitivity of cancer cells to anticancer drugs. These results suggested that ANT2 is a key player in tumor progression and a promising target for cancer therapy. However, the tumor-supportive mechanisms of ANT2 are still poorly understood. Our previous study indicated that ANT2 is a novel repressor of STING signaling pathway. Therefore, the goal of this project is to investigate the role of STING repressor-ANT2 in chemotherapy induced cell death and clarify the novel chemoresistance mechanism of ANT2. **Progress and results** 1) By using *in vitro* cell model and *in vivo* mice model, we found that chemotherapy(Doxorubicin, Cisplatin and Etoposide) treatment induces the expression of interferons(IFNs) and cytokines (by using qRT-PCR and ELISA detection), and this induction is dependent on cGAS-STING signaling pathway (by using cGAS/STING knockdown cell line or STING KO mice). An elevatus expression of interferons(IFNs) and cytokines are observed in ANT2 knock out cells or mice upon chemotherapy treatment and cause a more serious cell death compare with wild type. ANT2 is induced by chemotherapy treatment *in vitro* and *in vivo* (not only mRNA level but also protein level) but this induction is not dependent on STING pathway and also p53

	pathway. ANT2 interacts with STING and represses its downstrean signaling transduction. 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., 2018 is in preparation. **Remaining Work and Expected Results** 1) The mechanism of ANT2 induction upon chemotherpy is still unknow. To fullly understand this, we will analyze the promoter of ANT2 and identify the factors involved in the induction of ANT2. 2) To fully understand the mechanism of ANT2 repressing STING signaling pathway and STING-mediated innate immune response, we will clarify the role ANT2 on STING-cGAMP interaction. Due to the interaction site of ANT2 with STING is overlap with cGAMP binding site, therefore, we specurate that ANT2 may inhibit cGAMP binds to STING.
Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】
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