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ROLE OF CYCLOOXYGENASE 2 (COX-2) IN LIVER MITOCHONDRIAL FUNCTION AFTER ISCHEMIA/REPERFUSION DAMAGE

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Background: Adult hepatocytes fail to induce cyclooxygenase 1 (COX-2) expression, the key enzyme in the synthesis of prostaglandins, regardless of the pro-inflammatory factors used. COX-2 expression is restricted to those situations in which dedifferentiation or proliferation occur. In parallel using mice genetically modified to selectively express human COX-2 (hCOX-2-Tg) in hepatocytes [Protective Role of Hepatocyte Cyclooxygenase-2 Expression Against Liver Ischemia-Reperfusion Injury in Mice. Motiño et al. Hepatology. 2019 Aug;70(2):650-665. doi: 10.1002/hep.30241], we have demonstrated an increased tolerance to ischemia-reperfusion injury (IRI) with an increased functional recovery, a diminished cellular necrosis and less inflammation. It is known that mitochondria have a major role in IRI damage by increasing oxidative stress, decoupling metabolic state, and inducing apoptosis. In this work, we analysed different aspects in order to characterize the impact of COX-2 in mitochondrial function after a 90 min ischemia followed by 4h of reperfusion. **Methods:** We performed high-resolution respirometry with isolated mitochondria and liver homogenates from hCOX-2-Tg, as well as we analyzed the expression of different components of the electron transport chain supercomplexes, mitochondria morphology by transfer electron microscopy, fusion/fission events and gene or protein expression involved on mitochondria function. **Results:** Our overall results suggest that hCOX-2-Tg mice-derived mitochondria are more active than wild-type-derived mitochondria, apparently because of a higher contribution from fatty acids metabolism and complex I subtrates. **Conclusion:** These data suggest a new link between COX-2 and mitochondria, which might contribute to the protective effects of COX-2 against IRI.

Disclosures:

The following people have nothing to disclose: Marina Fuertes-Agudo, Carme Cucarella, Rocío Brea Contreras, Lisardo Boscá, Paloma Martin-Sanz, Marta Casado Pinna

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SCREENING OF ADAM9 INHIBITORS FOR SUPPRESSION OF SOLUBLE MICA IN HEPATOCELLULAR CARCINOMA

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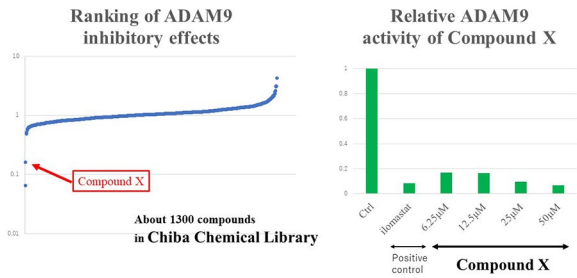
Background: Our previous genome-wide association study (GWAS) revealed that the single nucleotide polymorphisms in MHC-class I-related chain A molecule (MICA) was a risk factor for hepatocarcinogenesis [Nat. Genet. 2011]. MICA is a NKG2D ligand expressing on hepatocellular carcinoma (HCC) to promote cancer immunity by NK cell. However, A disintegrin and metalloprotease 9 (ADAM9), which is upregulated in HCC, inhibits the immunoreaction to cleave MICA on HCC. Moreover, the cleaved MICA in serum suppresses the activity of NK cell directly. Therefore, it is important to suppress ADAM9 in HCC for activating the cancer immunity. Then we searched compounds inhibiting ADAM9 to develop the therapy for activating cancer immunity in HCC. **Methods:** Screening of inhibitors of ADAM9 was performed by using fluorescence resonance energy transfer (FRET) assay with Chiba Chemical Library (CCL) as the screening compound library consisting of 1,300 novel compounds. Hit compounds were determined by the criteria (fluorescence intensity < 0.6). The effects of the hit compounds on soluble MICA production were confirmed by ELISA of supernatants derived from HCC cell lines (HepG2 and PLC/PRF/5). Cell viability assays were also performed to examine cytotoxicity of the compounds. The effect of soluble MICA on NK cells were analyzed using flow cytometry and co-culture to explore the significance of soluble MICA inhibition in anticancer therapy. **Results:** A screening method using FRET assay resulted in identification of 5 compounds based on the criteria mentioned above. Among them, polycyclic compound, Compound X exhibited the lowest fluorescent signal strength, indicating a strong inhibitor of ADAM9 candidate. The results of ELISA revealed that the supernatant derived from Compound X-treated HCC cells significantly decreased soluble MICA with low compound concentration. Cell viability assays demonstrated that there was less observational cytotoxicity with Compound X. When the recombinant MICA was added, NKG2D on NK cells were downregulated. Co-culture experiments with NK cells and hepatoma cells in which NKG2D was suppressed by recombinant MICA showed that the cytotoxicity of NK cells was decreased. Therefore, by reducing soluble MICA, Compound X might be a therapeutic agent for HCC. **Conclusion:** Compound X might be a potential therapeutic agent for HCC through the inhibition of ADAM9-mediated soluble MICA production.



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