ABSTRACT

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Thesis title: Identification and validation of Sirtuin 3 as a new common target of non-steroidal anti-inflammatory drugs (NSAIDs) to induce gastric mucosal injury and gastric adenocarcinoma cell death

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Non-steroidal anti-inflammatory drugs (NSAIDs), the indispensable medicines to treat pain and inflammation are presently also repurposed to treat cancers, thereby greatly increasing their pharmacological utility. However, severe gastrointestinal complications due to long-term NSAID treatment also discourage their safe usage while warranting precise identification of their molecular targets and mode of action. Cyclooxygenase-independent toxic effects of NSAIDs on mitochondria leading to apoptosis is appreciated; although, precise sub-mitochondrial target/s of these drugs controlling cellular bioenergetics and metabolic integrity is yet elusive. The present study, therefore, used a forward approach of differential global gene expression profiling by high-depth next-generation transcriptome sequencing of untreated and indomethacin (representative cyclooxygenase-non selective NSAID)-treated rat gastric mucosa as well as human gastric adenocarcinoma (AGS) cells to identify any putative common target of NSAIDs involved in controlling mitochondrial dysfunction and cell death. Contextually, mitochondrial NAD^+-dependent class III histone deacetylase sirtuin 3 (SIRT3), commonly referred to as 'mitochondrial metabolic guardian', was identified as a hitherto unreported NSAID target which was severely downregulated to activate cytotoxic pathways leading to apoptosis both in the normal gastric mucosa as well as in AGS cells. Isothermal calorimetry-based direct interaction analysis followed by deacetylase activity assay clearly revealed that indomethacin competitively inhibited SIRT3 owing to a binding-site overlap with NAD. It was clearly observed that indomethacin, by down-regulating SIRT3 upstream regulators like PGC1α and ERRα as well as reducing APMK activation, severely reduced SIRT3 expression to trigger mitochondrial proteome hyperacetylation and specific acetylation of mitochondrial base excision repair enzyme (OGG1) and mitochondrial superoxide dismutase (SOD2). Indomethacin actually blocked the AMPK/PGC1α/SIRT3 signaling axis resulting in the induction of severe mitochondrial oxidative stress. This further led to mtDNA damage, downregulation of ETC complex genes, mitochondrial fragmentation and elevated mitophagy, ultimately causing cell death. Functional validation of the observed data involved pre-treatment of the rats with honokiol (specific pharmacological inducer of SIRT3) or silencing of SIRT3 expression in the AGS cells followed by indomethacin in either case. In the rat gastric injury model, honokiol pre-treatment resulted in significant prevention of mitochondrial pathology, inflammatory signaling and hence gastric mucosal damage without affecting basal gastric acid secretion, unlike standard anti-ulcer drug lansoprazole. On the other hand, in the *in vitro* gastric cancer model in AGS cells, SIRT3 knockdown severely aggravated NSAID-induced mitochondrial dysfunction as well as deteriorated cell cycle progression to increase cancer cell death. Interestingly, SIRT3 reduction was found to be a common response elicited by other popular NSAIDs like diclofenac, aspirin and ibuprofen which are rampantly used for human clinical purposes. Altogether, the study for the first time identified and established SIRT3 as a predominant target of NSAID which gets severely downregulated in a cyclooxygenase-independent pathway leading to cell death both in the normal gastric mucosa as well as in gastric adenocarcinoma. While precisely exploiting specific pharmacological upregulation of endogenous SIRT3 may certainly mitigate the gastrotoxicity of NSAIDs during inflammation management, NSAID treatment coupled with targeted SIRT3 depletion may prove highly efficient in the management of drug-resistant tumors combinatorial chemotherapy approach.

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