"Targeting Mitochondrial Dynamics and Mitophagy in Gastrointestinal Carcinomas: Unraveling the Therapeutic Potential of Drp1, Opa1, and PINK1 Pathways with NSAIDs and Conventional Anti-Cancer Drugs"

Project Summary:

Despite Warburg's hypothesis, mitochondria play diverse roles in the advancement of many malignant tumors by affecting the cell cycle, gene expression, metabolism, immune response, cell proliferation, and survivability. Furthermore, enhanced intra-mitochondrial reactive oxidant production plays a significant part in the cancer microenvironment. Mitochondria are architecturally dynamic organelles, that undergo cycles of fission and fusion and therefore have a pivotal role in mtDNA dispersal, cristae reformation, bio-energetic distribution, mitochondrial turnover maintenance, and numerous other processes. GTPases like Mitofusin 1 (Mfn1), Mfn2, and optic atrophy 1 (Opa1), are largely responsible for mitochondrial fusion, whereas GTPase dynamin-related protein 1 (Drp1) has been accountable for mitochondrial fission. Tumor suppressor p53 modulates Opa1 activation and mitochondrial disintegration, assisting in the proliferation of ovarian and cervical cancer cells. Drp1 expression and activity, on the contrary, have been correlated to increased glycolytic metabolism in lung cancer, as well as active oxidative metabolism and mitochondrial fusion in several metastatic cancer models. Surprisingly, the repercussions of Drp1 intensity of expression depend on the cancer type. for instance, Drp1 knockdown increases both oxygen consumption rate (OCR) and ATP generation in Ras-transformed MEFs cells. Drp1 knockdown, on the other hand, lowers OCR in HEK-TtH cells, inhibiting cell proliferation. Autophagy and mitochondrial autophagy, or mitophagy, exert dual roles in cancer, acting as either pro-oncogenic or tumor suppressors. PINK1, a mitophagy arbitrator, becomes elevated in squamous cell carcinoma but minimized its function in ovarian cancer. The concurrent function of these proteins (Drp1, PINK1, etc.) in the context of cancer advancement allows them to be an essential consideration for oncology investigations to uncover predicted case-specific therapeutic avenues. In this present endeavor, I want to investigate the significant contribution of Drp1, Opa1, and PINK1 as possible avenues for therapeutic targets in signaling pathways that regulate mitochondrial metabolism, in addition, have been linked to cancer cell proliferation in the context of digestive tract carcinomas (e.g., gastric adenocarcinoma, hepatocellular carcinoma, etc.). The vicious relationship of mitochondrial dynamics-mitophagy-cell proliferation will be investigated using chemical induction/inhibition in addition to siRNA-mediated knock-down in normal physiological circumstances along with under the influence of some traditional (e.g., Cisplatin,

doxorubicin) and unconventional (e.g., NSAIDs) anti-cancer drugs. Our earlier research along with additional investigations have confirmed the function of NSAIDs to induce mitochondrial dysfunction and given evidence of possible anti-cancer benefits. However, the precise process is yet unknown. From a molecular standpoint, this study will attempt to investigate the efficacy of the pathway used by NSAIDs to demonstrate anti-neoplastic actions in gastrointestinal carcinomas.

Objectives:

Objective 1: Dynamic investigation of the cellular decision point and key actors engaged in triggering programmed cell death machinery in the context of mitochondrial pathology.

Objective 2: The involvement of mitochondrial morpho-dynamic facilitators in cell fate determination is going to be investigated using different pharmacophoric suppression and/or activation of the fission-fusion and subsequent mitophagy pathways to demonstrate how they communicate with programmed cell-death machinery.

Objective 3: Towards a deeper understanding, the impact caused by NSAID administration on the bioenergetic state of cancer cells in comparison to conventional anti-neoplastic drugs were studied, in addition to the pharmacological modification of glucose utilization.

Keywords:

Mitochondria, Drp1, Opa1, fission-fusion, mitophagy, PINK1, gastrointestinal carcinoma

Expected Output and Outcome of the Proposal:

Expected outcome 1. Determination of the unique cellular decision point and critical actors involved in the progression of ETC (electron transport chain)-mediated mitochondrial pathology to cancer cell death.

Expected outcome 2. Recognition of the vital function of Drp1, PINK1, and Opa1 for recognizing the degree of mitochondrial impairment and instructing cells towards apoptosis in the presence of indomethacin, which may provide innovative non-canonical and/or multimodal strategy for cancer remedies.

Expected outcome 3. We will also gain a better understanding of the role of glucose and energy metabolism in the progression of cancer cells.

The findings of this investigation will provide clarity on how those are altered in the event of NSAID medication and the possibility that they might be used as therapeutic targets. Expected outcome/importance Cancer is the leading cause of death and morbidity in the globe. The process through which mitochondrial dynamics and mitophagy become altered in malignancies, particularly in a tissue-specific way, remains unanswered. Despite numerous research efforts, no viable therapy based on signaling to portray underlying mitochondrial morphological dynamics and mitophagy has been offered. This suggests that pharmacophores/drugs can be used in an unorthodox anti-cancer therapy approach that focuses on novel pathways that can induce programmed cell death.

Technical Document:

1. **Modern technology at the forefront**

Cancer remains a worldwide threat, according to the World Health Organisation projecting 18.1 million new cases and 9.6 million deaths in 2018. Under the India Council of Medical Research (ICMR), over 1300 Indians die from cancer every single day. Every year, roughly a million new cancer cases are identified, including a significant mortality rate (more than 17% in 2014).

Mitochondria are organelles that synthesize ATP and supply a significant portion of the energy in the majority of cells (1). Alteration in cancer cell metabolism and the importance of mitochondrial integrity towards providing the bio-energetic demands for the development of cells goes beyond Warburg's orthodox ideology (2,3). Mitochondria modulate cell cycle, gene expression, metabolism, immune response, cell survival, and other elements of cancer cell proliferation to play multidimensional roles in diverse malignant tumor stages. Furthermore, under numerous clinical situations, mitochondrial oxidative stress (MOS) and subsequent apoptosis harm multiple organs (4). Elevated intra-mitochondrial reactive oxidant production, comprising superoxide (O2•), hydrogen peroxide (H2O2), and hydroxyl radical (•OH), plays an important role in the cancer milieu. Mitochondria represent a structurally dynamic organelle that undergoes cycles of fission and fusion which serve a substantial part in mtDNA nucleoid dispersal, cristae reformation, bio-energy distribution, mitochondrial turnover maintenance, and so on (5,6). GTPases, mitofusin 1 (Mfn1), Mfn2, and optic atrophy 1 (Opa1), are largely responsible for mitochondrial fusion, whereas GTPase dynamin-related protein 1 (Drp1), mitochondrial

fission factor (MFF) are responsible for mitochondrial fission. Whenever the activity of these GTPases is impaired, mitochondrial dynamics and cellular metabolism are altered.

In tumors, mitochondrial metabolism and structure interplay in a complicated way. Tumor suppressor p53 is implicated in Oma1-mediated L-Opa1 processing and mitochondrial dispersion, both of which help in the proliferation of ovarian and cervical cancer cells (7). Opa1 suppression reduces OCR and intracellular ATP. Drp1 expression and exertion, on the contrary, have been connected to increased glycolytic metabolism in many types of cancer, including lung cancer (8), and active oxidative metabolism has also been linked to mitochondrial fusion in some metastatic cancer models, including pancreatic cancer (9). Hepatocellular carcinoma, breast cancer, lung cancer, thyroid cancer, and glioblastoma have all been shown to have elevated levels of Drp1 expression (9). Contrary to this, investigations have revealed that Drp1 is involved in the Bax/Bak-dependent permeabilization of the mitochondrial outer membrane, which is required for apoptosis (10). Surprisingly, the impact of Drp1 expression level varies with cancer type. Drp1 knockdown, for instance, increases both oxygen consumption rate (OCR) and ATP generation in Ras-transformed MEFs and SK-MEL-28 cells (11). Drp1 knockdown, on the other hand, lowers OCR in HEK-TtH cells (12), inhibiting cell proliferation. Autophagy and mitochondrial autophagy, or mitophagy, serve dual functions in cancer, acting as either pro-oncogenic or tumor suppressors (13). PINK1, a mitophagy arbitrator, becomes elevated in squamous cell carcinoma but downregulated in ovarian cancer (14). The concurrent function of these proteins (Drp1, PINK1, etc.) in the event of cancer progression makes them a crucial choice for oncology investigations to uncover likely case-specific therapeutic targets.

Mdivi-1 is commonly utilized as a Drp1 and mitochondrial fission inhibitor to investigate the metabolic significance of fission. Interventions with mdivi-1, inconsistently raised and lowered OCR depending on different cell types. Typically, mdivi-1 therapy boosts mitochondrial fusion and activity, therefore improving OCR rates. Conversely, mdivi-1 treatment MDA-MB-231 cells and H1299 cells greatly reduce OCR without modifying the cellular ATP level. Additionally, a Drp1-independent function for mdivi-1 as a mitochondrial complex I inhibitor, inducing ROS and suppressing OCR is also proposed. Mdivi-1 dysregulates mitochondrial bioenergetics in high oxidative oestrogen receptor-positive breast cancer, increasing glycolysis without changing mitochondrial structure. As a whole, mdivi-1 can be utilized to investigate cancer metabolism as well as propagation to

find new treatment routes. Additional pharmacophores relevant in investigating the route include 1'10-phenanthroline, which stimulates both fission and mitophagy. To investigate the nexus, general autophagy inhibitors such as Bafilomycin, 3- Methyladenine can be utilized.

References:

1. Zhao, J., Zhang, J., Yu, M., Xie, Y., Huang, Y., Wolff, D. W., Abel, P. W., and Tu, Y. (2013) Mitochondrial dynamics regulates migration and invasion of breast cancer cells. Oncogene 32, 4814-4824

2. Jezierska-Drutel, A., Rosenzweig, S. A., and Neumann, C. A. (2013) Role of oxidative stress and the microenvironment in breast cancer development and progression. Advances in cancer research 119, 107-125

3. Chen, H., and Chan, D. C. (2017) Mitochondrial Dynamics in Regulating the Unique Phenotypes of Cancer and Stem Cells. Cell metabolism 26, 39-48

4. Rubattu, S., Pagliaro, B., Pierelli, G., Santolamazza, C., Castro, S. D., Mennuni, S., and Volpe, M. (2014) Pathogenesis of target organ damage in hypertension: role of mitochondrial oxidative stress. International journal of molecular sciences 16, 823-839

5. Westermann, B. (2012) Bioenergetic role of mitochondrial fusion and fission. Biochimica et biophysica acta 1817, 1833-1838

6. Ishihara, T., Ban-Ishihara, R., Maeda, M., Matsunaga, Y., Ichimura, A., Kyogoku, S., Aoki, H., Katada, S., Nakada, K., Nomura, M., Mizushima, N., Mihara, K., and Ishihara, N. (2015) Dynamics of mitochondrial DNA nucleoids regulated by mitochondrial fission is essential for maintenance of homogeneously active mitochondria during neonatal heart development. Molecular and cellular biology 35, 211-223

7. Zhao, X., Tian, C., Puszyk, W. M., Ogunwobi, O. O., Cao, M., Wang, T., Cabrera, R., Nelson, D. R., and Liu, C. (2013) OPA1 downregulation is involved in sorafenib-induced apoptosis in hepatocellular carcinoma. Laboratory investigation; a journal of technical methods and pathology 93, 8-19

8. Rehman, J., Zhang, H. J., Toth, P. T., Zhang, Y., Marsboom, G., Hong, Z., Salgia, R., Husain, A. N., Wietholt, C., and Archer, S. L. (2012) Inhibition of mitochondrial fission

prevents cell cycle progression in lung cancer. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 26, 2175-2186

9. Dai, W., and Jiang, L. (2019) Dysregulated Mitochondrial Dynamics and Metabolism in Obesity, Diabetes, and Cancer. Frontiers in endocrinology 10, 570

10. Cassidy-Stone, A., Chipuk, J. E., Ingerman, E., Song, C., Yoo, C., Kuwana, T., Kurth, M. J., Shaw, J. T., Hinshaw, J. E., Green, D. R., and Nunnari, J. (2008) Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. Developmental cell 14, 193-204

11. Serasinghe, M. N., Wieder, S. Y., Renault, T. T., Elkholi, R., Asciolla, J. J., Yao, J. L., Jabado, O., Hoehn, K., Kageyama, Y., Sesaki, H., and Chipuk, J. E. (2015) Mitochondrial division is requisite to RAS-induced transformation and targeted by oncogenic MAPK pathway inhibitors. Molecular cell 57, 521-536

12. Kashatus, J. A., Nascimento, A., Myers, L. J., Sher, A., Byrne, F. L., Hoehn, K. L., Counter, C. M., and Kashatus, D. F. (2015) Erk2 phosphorylation of Drp1 promotes mitochondrial fission and MAPK-driven tumor growth. Molecular cell 57, 537-551

13. Kocaturk, N. M., Akkoc, Y., Kig, C., Bayraktar, O., Gozuacik, D., and Kutlu, O. (2019) Autophagy as a molecular target for cancer treatment. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 134, 116-137

14. Vara-Perez, M., Felipe-Abrio, B., and Agostinis, P. (2019) Mitophagy in Cancer: A Tale of Adaptation. Cells 8

2. Origin of the proposal

Scientific Reasoning and Hypotheses:

From a molecular standpoint, this study will attempt to investigate the efficacy of the pathway used by NSAIDs to demonstrate anti-neoplastic actions in gastrointestinal tract carcinomas. Mitochondrial dynamics and mitophagy proteins act erratically in many cancer cells. As a result, pharmacological inhibition/induction of the relevant pathways will have particular anti-proliferative effects that will be effective in cancer therapy.

Importance:

Cancer is the leading cause of death and morbidity in the globe. The method by which mitochondrial dynamics and mitophagy are changed in cancer, particularly in a tissue-specific way, remains a mystery. Despite numerous research efforts, no viable therapy based on signaling acting underlying mitochondrial morphological dynamics and mitophagy has been offered so far. This suggests that pharmacophores/drugs can be used in an unorthodox anti-cancer therapy paradigm that focuses on new targets that can induce programmed cell death. Aside from cancer, the implications of this discovery might be far-reaching because mitochondrial dynamics and mitophagy proteins have been connected to aging-related disorders such as neurodegeneration. As a result, a thorough investigation of these pathways and the development of effectively manipulative medications may be therapeutically useful as potential therapeutic strategies to combat additional aging-related progressive, degenerative disorders.

1. Strategy for Research

Objective 1: Dynamic investigation of the cellular decision point and key actors engaged in triggering programmed cell death machinery in the context of mitochondrial pathology.

Detailed Methodology for Objective 1: (1st 8 Months):

The convoluted signaling cross-talk of mitochondrial redox status, dynamics, and autophagy in determining cell cycle state and cell fate will be examined chronologically in this experiment by the infusion of the unconventional mitochondrial complex I antagonist indomethacin (an NSAID).

I. To examine digestive tract carcinomas, gastric adenocarcinoma cell AGS and hepatocellular carcinoma cell HepG2 will be evaluated. In a time-point assessment, Indomethacin would produce mitochondrial pathology in contrast with additional mito-pathology inducer drugs for cancer like doxorubicin.

II. Mitochondrial metabolic parameters and energy expenditure will be examined following medication therapy using the respiratory control ratio (RCR), membrane potential analysis, cardiolipin, and ATP content assessment. OXPHOS complex assessments will be done at several periods after treatment to measure the extent of ETC complex suppression, as previously demonstrated by indomethacin inhibition of complex I.

III. Kinetic variations in mitochondrial integrity will be correlated with cell cycle modifications, cell viability by MTT assay, and apoptosis/necrosis analysis by

flow cytometry to establish the start point of cell apoptosis pathways. The characteristics of the death signaling will also be evaluated by measuring differential activation of Caspases 3, 9, and other death markers HMGB1, RIPK3, etc.

IV. The mitochondrial cytopathologic status will be compared with other ETC complex I inhibitors, such as rotenone, haloperidol, and olanzapines, which are recognized to manipulate Electron transport chain (ETC) operation, to determine the necessity of complex I inhibition for indomethacin's anti-proliferative effect.

V. The activation of mitochondrial dynamics regulators Opa1, Drp1, and mitophagy regulator PINK1 in response to indomethacin administration will be assessed using western blot and immune-fluorescence microscopy, which will correlate with the activation of crucial pro- or anti-proliferative signals.

VI. Mitochondrial oxidative stress will be evaluated. Mitochondrial DNA damage (by PCR) and 8-Oxo-dG level estimate (by ELISA) will be assessed, followed by quantitative assessments of mitochondrial protein carbonylation, lipid peroxidation, and glutathione depletion. Standard procedures will be used.

Objective 2: The involvement of mitochondrial morpho-dynamic facilitators in cell fate determination is going to be investigated using different pharmacophoric suppression and/or activation of the fission-fusion and subsequent mitophagy pathways to demonstrate how they communicate with programmed cell-death machinery.

Detailed Methodology for Objective 2: (2nd 8 Months):

After assessing the endogenous signaling of dynamics and mitophagy, we will manipulate these two types of machinery using the fission inhibitor mdivi, the fission and mitophagy promoter 1'10-phenanthroline, the autophagy inhibitor Bafilomycin, and 3-Methyladenine in the presence/absence of indomethacin to see how it affects cell cycle and proliferation. The key participants in the pathways will be validated by gene alteration using targeted siRNA. Cellular redox state as a contributor to this pathway will also be assessed.

The precise design of evaluation sets to be included, and pharmacological combinations to be used will be determined by the outcomes of the initial objective which we hope to get throughout the first 8 months.

Objective 3: Towards a deeper understanding, the impact caused by NSAID administration on the bioenergetic state of cancer cells in comparison to conventional anti-neoplastic drugs were studied, in addition to the pharmacological modification of glucose utilization.

Detailed Methodology for Objective 3: (3rd 8 Months):

Considering glucose metabolism, and ETC efficiency are important in cancer development, we will investigate glucose and energy metabolism in cancer cells exposed to Indomethacin. Selective increase of ETC over glycolysis by hydrogen-rich saline or glycolysis suppression by 2-deoxyglucose or 3-bromopyruvate (3-BrPA) can help comprehend mitochondrial respiratory proficiency and hypoxia in the presence/absence of indomethacin.

The detailed design of experiment sets to be included, and pharmacological combinations to be used will be determined by the findings of objectives 1 and 2 that we hope to get throughout the first 6 months.

Timelines (Month wise plan of work):

Activity/Deliverable	**4**	**8**	**12**	**16**	**20**	**24**
Aim 1: Dynamic investigation of the cellular decision point and key actors engaged in triggering programmed cell death machinery in the context of mitochondrial pathology.						
Aim 2: The involvement of mitochondrial morpho-dynamic facilitators in cell fate determination is going to be investigated using different pharmacophoric suppression and/or activation of the fission-fusion and subsequent mitophagy pathways to demonstrate how they communicate with programmed cell-death machinery.						
Aim 3: Towards a deeper understanding, the impact caused by NSAID administration on the						

bioenergetic state of cancer cells in comparison to conventional anti-neoplastic drugs was studied, in addition to the pharmacological modification of glucose utilization.						

Preliminary Data:

A. Establishment of mitochondrial dysfunction by treatment of indomethacin in gastric cancer cells:

Mitochondria modulate cell cycle, gene expression, metabolism, immune response, cell proliferation, and viability in several types of cancers. We used Indomethacin to inhibit mitochondrial Complex I activity and assessed cell survival to determine the significance of mitochondrial integrity in gastric adenocarcinoma cells (AGS). The dose-response curve from the MTT-reduction experiment (Fig 1A) clearly showed reduced cell viability, which was confirmed by a time-dependent reduction in cell viability followed up to 48 hours (Fig 1B). The 50% inhibitory concentration (IC_{50} dose) of 500 µM of indo was used for subsequent experiments until otherwise mentioned with an optimum incubation of 24 hours. The predicted result of indomethacin therapy was confirmed by a significant decrease in mitochondrial complex I activity (Fig 1C). Mitochondrial functional integrity was found to be severely compromised, as evidenced by decreased mitochondrial respiration or Respiratory Control Ratio (RCR, calculated as a ratio of state 3 and state 4 respirations), indicating a mitochondrial respiratory defect likely caused by dysfunctional complex I of the Electron Transport Chain (ETC) and culminating in decreased ATP content (Fig 1C). Furthermore, decreased mitochondrial membrane potential ($\Delta\Psi m$) showed mitochondrial depolarization, most likely due to an indo-induced ETC leak at complex I (Fig 1C).

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Figure 1

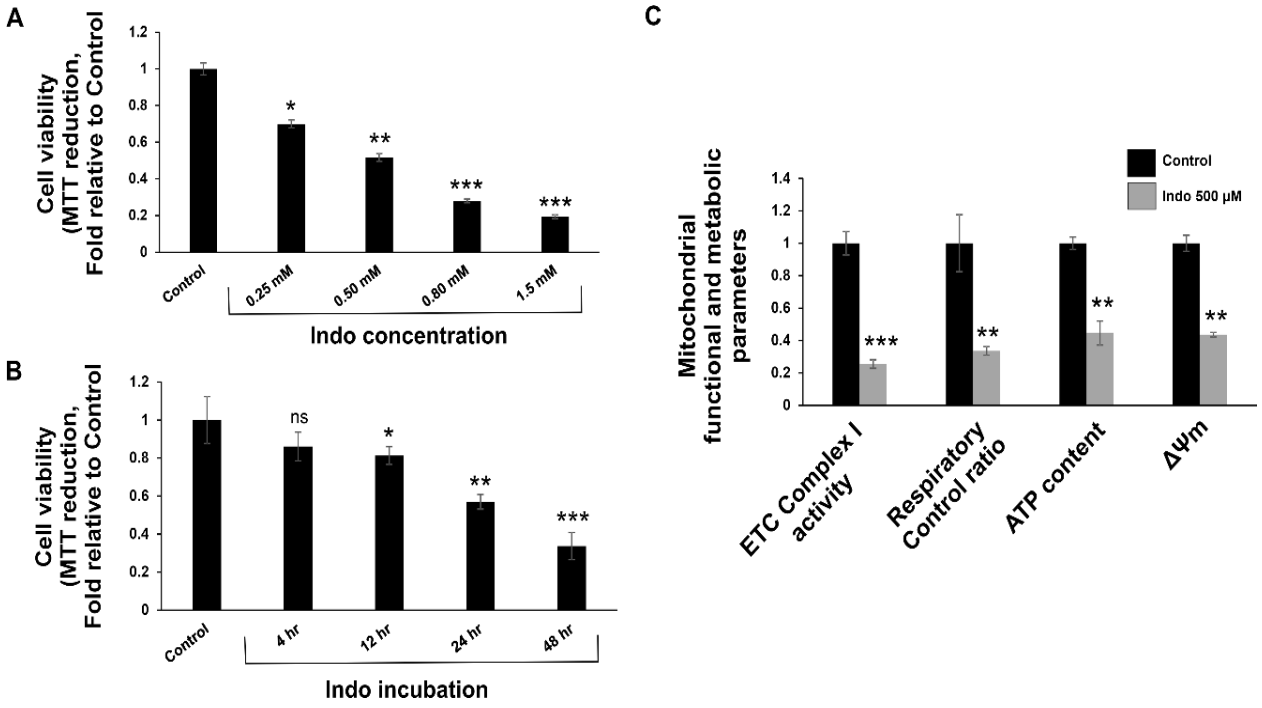

Figure 1: Evaluation of the effect of Mitochondrial complex I inhibitor indomethacin (this onwards "indo") on human gastric adenocarcinoma cell, AGS. A) Evaluation of dose-dependent alteration of cell-viability by MTT-reduction assay. B) Evaluation of time dependent alteration of cell-viability by MTT-reduction assay. C) Mitochondrial functional and metabolic parameters in the form of ETC Complex I activity, Respiratory control ratio (RCR), ATP content and mitochondrial trans-membrane potential (ΔΨm) were analyzed after 0.5 mM (IC50 dose) indo treatment for 24 hours. Results were presented as fold relative to control. Error bars, mean ± SD. * $P < 0.05$; **$P < 0.01$, ***$P < 0.001$, versus control; ##$P < 0.01$versus indomethacin, ns = not significant as calculated by ANOVA followed by Bonferroni's post hoc test.

B. Mitochondrial complex I inhibition accompanied by blocking of mitochondrial fission in combination aggravates gastric cancer cell death:

Electron leak is the evident outcome of defective ETC, which logically induces reduced mitochondrial bioenergetics and functional integrity, which is known to trigger structural modifications. Mitochondria, being a morphologically dynamic organelle, can alter its form in response to environmental cues. Many malignancies adopt the fused network structure to diffuse defective foci with the assistance of neighboring functioning counterparts. On the contrary, many cancer kinds become fragmented to be cleaned out or to keep up with the high proliferation rate. We suppressed fission using the mitochondrial fission inhibitor Mdivi-1 and found a dose-dependent reduction in cell viability in the MTT experiment (Fig 2A). We then sought to see if there was any synergism or antagonism in cells with complex I impairment (through indo therapy) and inhibited fission (by mdivi-1). A suboptimal dosage of 250 µM indo was chosen for the treatment combination experiment to highlight small differences. Interestingly, the doubly-compromised cells lost viability when 75 µM mdivi-1 was combined with 250 µM indomethacin, indicating a considerable increase in cell mortality when compared to treatment with either of the medications alone (Fig 2A-B). Interestingly, mdivi-1 administration did not affect the viability of normal gastric epithelial cells (Fig 2C). These findings imply that in AGS cells, mitochondrial fission aids in the development and

proliferation of gastric cancer cells, and it also plays an important role in the case of environmental insults that cause mitochondrial damage.

Figure 2

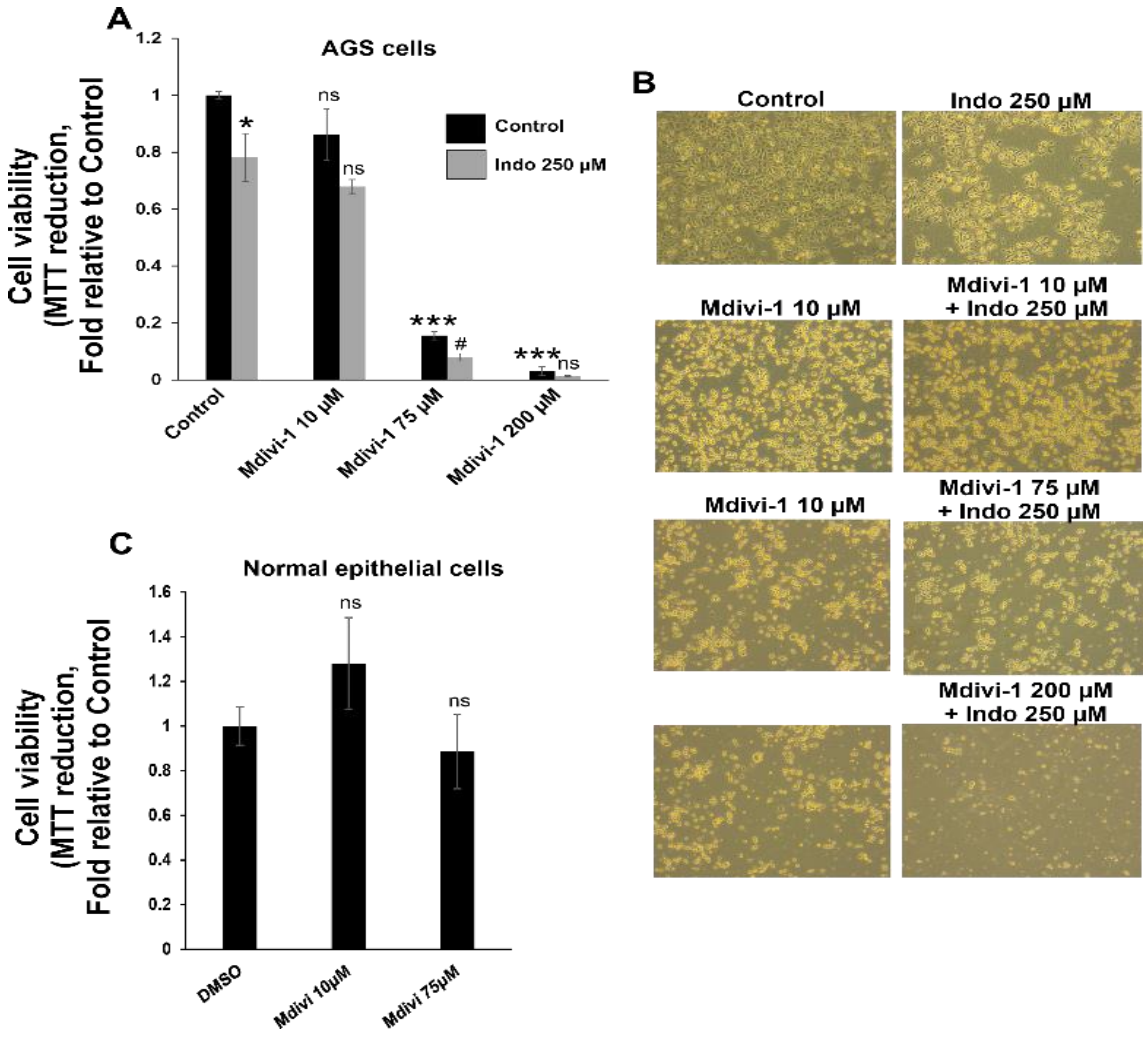

Figure 2: Assessment of the combinatorial effect of Mitochondrial complex I inhibition and fission blocking. A) The evaluation of cell viability by MTT reduction upon simultaneous treatment of indo and Mdivi-1. B) Phase-contrast images of AGS cells upon simultaneous treatment indo at 250 µM and Mdivi-1 at different concentrations. C) Effect of inhibition of mitochondrial fragmentation by mdivi-1 on normal gastric epithelial cells. Error bars, mean ± SD. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$, versus control; # $P < 0.05$ versus mdivi-1, ns = not significant as calculated by ANOVA followed by Bonferroni's post hoc test.

Impact of the research in the advancement of knowledge or benefit to mankind

This research significantly advances our understanding of gastric adenocarcinoma (GC) and offers promising therapeutic strategies that could benefit mankind. GC, being one of the most lethal forms of cancer, poses a substantial global health challenge with limited effective treatment options. The study's identification of mitochondrial dynamics, particularly the

downregulation of PINK1 and the role of Drp1, as crucial factors in the survival and proliferation of GC cells opens new avenues for targeted cancer therapy. By demonstrating that the combination of mitochondrial disruption (via Indomethacin) and the inhibition of mitochondrial fission (via Mdivi-1) leads to a significant reduction in GC cell viability, the research offers a novel approach to cancer treatment. The implications of this research extend beyond GC, as the mechanisms explored—mitophagy, mitochondrial fission, and the role of PINK1 and Drp1—are relevant to various other cancers. Thus, the study contributes to the broader field of oncology by identifying mitochondrial signaling as a potent therapeutic target. This could lead to the development of new drugs and treatment protocols that improve survival rates and quality of life for cancer patients, thereby offering substantial benefits to mankind in the fight against cancer.