Gallic acid mediated SIRT1 activation directs asymmetric mitochondrial fission followed by mitophagic flux inhibition to induce apoptotic cell death

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Abstract

Distinct mitochondrial fission parameters predict cancer cell survival and death owing to their fission site. Symmetric fission leads to cell survival with subsequent mitochondrial biogenesis; while, asymmetric fission leads to mitophagic clearance of dysfunctional mitochondria. The present study unveils imperative molecular regulation of SIRT1 (NAD-dependent protein deacetylase sirtuin-1), a class III HDAC as a critical controller of asymmetric mitochondrial fission. Gallic acid (GA), a small molecule activator of SIRT1, primarily induces DNM1Lmediated mitochondrial fission and perinuclear clustering with a subsequent decrease in the expression of RHOT1. Further, GA induced SIRT1 activation directs FIS1 recruitment to the asymmetric fission sites to the mitochondrial daughter filaments having low mitochondrial membrane permeability, higher mitochondrial superoxide, low mtDNA content, and high calcium efflux. In addition, our data showed that parkin was recruited to the asymmetrically fissioned mitochondrial sub-population to be engulfed by mitophagy. Mechanistically, the nuclear translocation of SIRT1 after gallic acid treatment redirects deacetylated LC3 from the nucleus to the cytoplasm to induce autophagy. Further, the induction of mitophagy followed by asymmetric mitochondrial fission is SIRT1 dependent as inhibition of SIRT1 blocks mitochondrial fission and mitophagy. Furthermore, GA impairs autophagic flux through decreased expression of RAB7A and its recruitment to lysosome and autophagosome. The subsequent accumulation of mitophagosome enhances generation of mitochondrial superoxide leading to apoptotic cell death in oral cancer cells.

Results

1. GA mediated mitochondrial perinuclear clustering is dependent on SIRT1

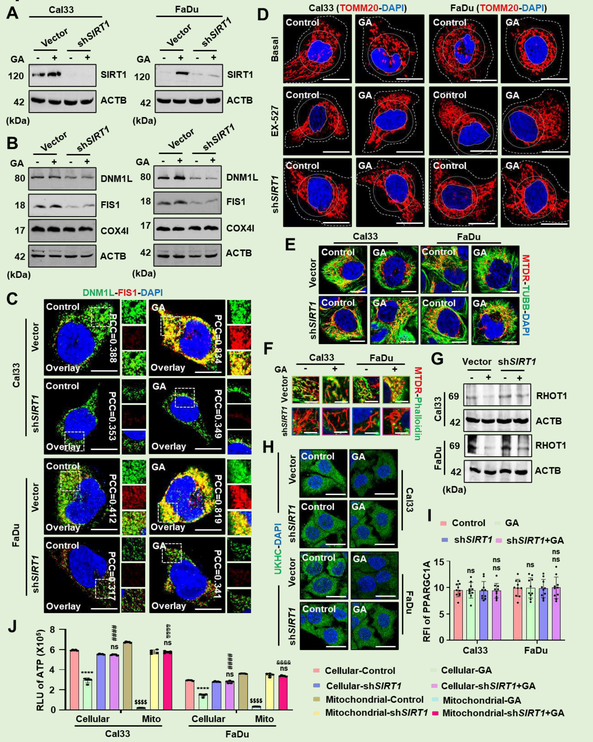
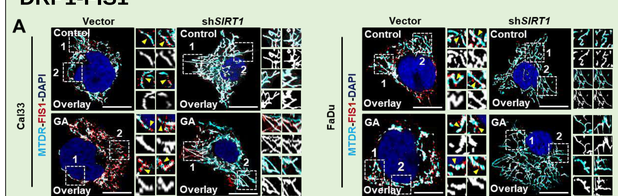


Fig.1. SIRT1 regulates perinuclear clustering of mitochondria: SIRT1 activation was evaluated in presence of GA (1A). Expression of DNM1L, FIS1 and COX4I was evaluated (1B). Colocalization of DNM1L-FIS1 (1C). Perinuclear clustering of mitochondria (1D). TUBB (1E) and phalloidin (1F) association with mitochondria. Expression of RHOT1 (1G) and UKHC (1H). PPARGC1A expression was measured by western blotting (1I). Activity of ATP was measured (1J).

2. SIRT1 is indispensable for GA-induced asymmetric mitochondrial fission in course of action with SIRT1-DRP1-FIS1



Statistical analysis: The mean ± SD was evaluated with two-way ANOVA and student's t-test. The p-value > 0.05 was considered not significant (ns), *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001 and ****p-value < 0.0001; #p-value was the comparison of treatment group with inhibitor-cotreated group. @p-value was the comparison of the inhibitor group with inhibitor-cotreated group.

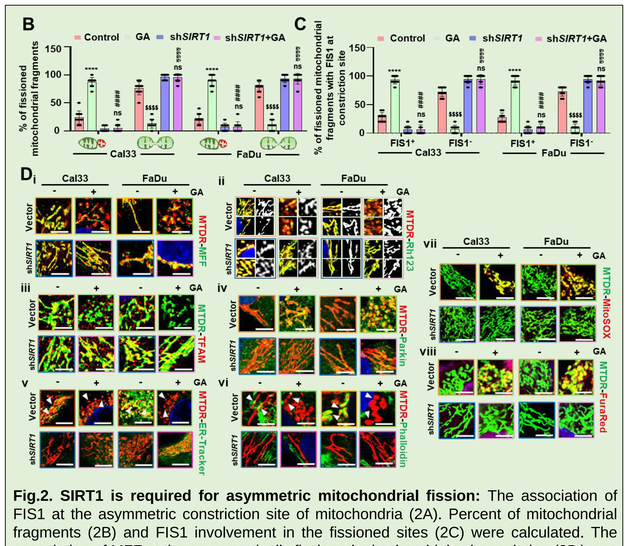


Fig.2. SIRT1 is required for asymmetric mitochondrial fission: The association of FIS1 at the asymmetric constriction site of mitochondria (2A). Percent of mitochondrial fragments (2B) and FIS1 involvement in the fissioned sites (2C) were calculated. The association of MFF at the asymmetrically fissioned mitochondrial subpopulation ($2D_i$) was measured in the presence of GA and sh*SIRT1* conditions. Further, the expression of Rhodamine 123 (D_{ii}), TFAM (D_{iii}), parkin (D_{iv}), ER-association with fissioned mitochondrial subpopulation (D_v), phalloidin association (D_{vi}), MitoSOX (Dv_{ii}) and calcium (D_{viii}) were measured in the presence and absence of SIRT1.

3. GA- induces mitophagy post asymmetric fission

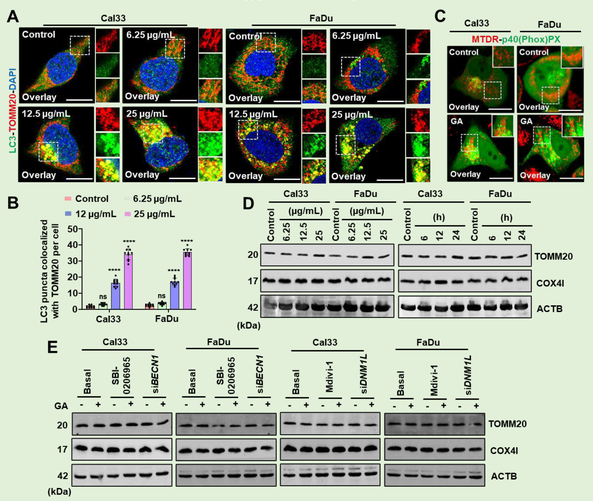


Fig.3. GA-induces mitophagy: The colocalization of LC3 and TOMM20 was done to check the mitophagosome formation in a dose dependent manner of GA treatment (3A and 3B). The The p40(Phox)PX was colocalized with MTDR to ckeck PI3KC3 activity around the fissioned mitochondria (3C). The expression of TOMM20 and COX4I were evaluated in a dose and time dependent manner (3D). Similarly, these preotein expressions were evaluated in the presence of autophagy inhibitors SBI-0206965 and si*BECN1*. In addition, these protein expressions were also checked in the presence of mitochondrial fission inhibitor Mdivi-1 and si*DNM1L* (3E).

4. SIRT1 regulated mitochondrial asymmetric fission is critical for mitophagy induction

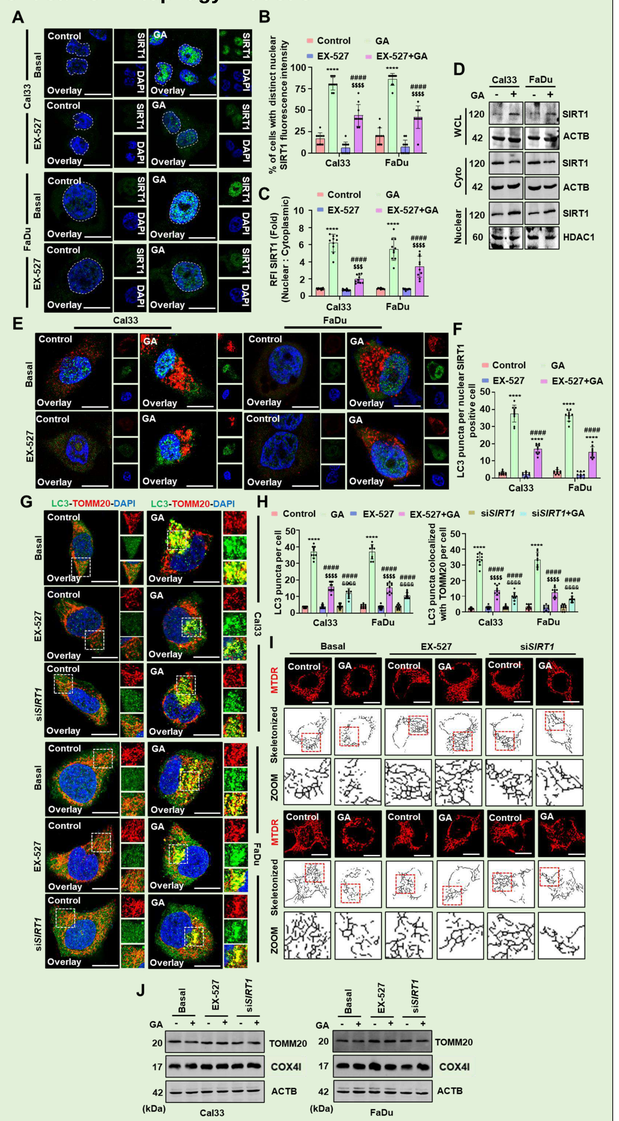


Fig.4. SIRT1 is critical for mitophagy induction: The nuclear translocation of SIRT1 is evaluated in the presence of GA and EX-527 (4A). The percentage of cells with nuclear SIRT1 (4B) and cytosol: nuclear ration of SIRT1 (4C) were calculated. The sub-cellular fractionation exhibited nuclear translocation of SIRT1 in presence of GA (4D). The LC3 puncta formation in cells with nuclear SIRT1 was further calculated (4E and 4F). The mitophagosome formation was evaluated by colocalizing LC3-TOMM20 in the presence of EX-527 and si*SIRT1* (4G).

The LC3 puncta and mitophagosome number (4H) were calculated. The mitochondrial skeleton analysis was done in the presence of EX-527 and si*SIRT1* (4I). The protein expression of TOMM20 and COX4I were evaluated in the presence of EX-527 and si*SIRT1* (4J)

5. Impaired mitophagic flux elicits mitophagosome accumulation leading to apoptosis

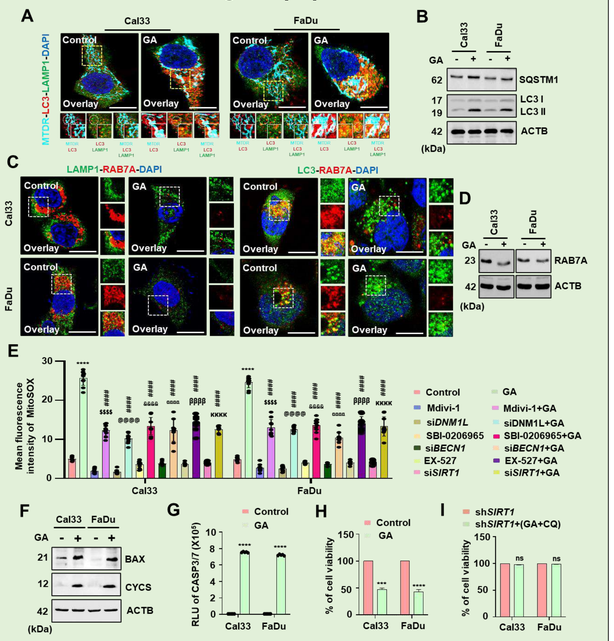
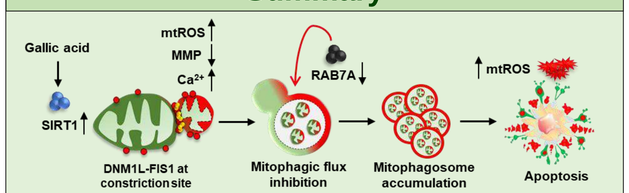


Fig.5. Mitophagic flux inhibition leads to mitophagosome formation for subsequent generates or mtROS to indue apoptosis: The colocalization of MTDR-LC3 and LAMP1 was done to check the status of mitophagosome formation (5A). The western blotting was done to check the protein expression of LC3 and SQSTM1 (5B). The recruitment of RAB7A to LAMP1 and LC3 was checked to confirm the impaired RAB7A recruitment (5C). The protein expression of RAB7A was evaluated by western blotting (5D). The MitoSOX activity was measured in the present of autophagy and fission inhibitors (5E). The expression of BAX and CYCS were measured by western blotting (5F). The CASP3/7 activity was measured (5G). The cell viability was checked by MTT assay (5H). In the absence of SIRT1 the cell death is rescued (5I).

Summary



Acknowledgement

We convey our thanks to the National Institute of Technology, Rourkela. SP acknowledges DST INSPIRE, Award reference number [IF180167], Department of Science and Technology, Government of India.