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A HYPOMETHYLATING TREATMENT INHIBITS AUTOPHAGY IN PANCREATIC CANCER CELLS AND INDUCES EFFECTIVE APOPTOSIS

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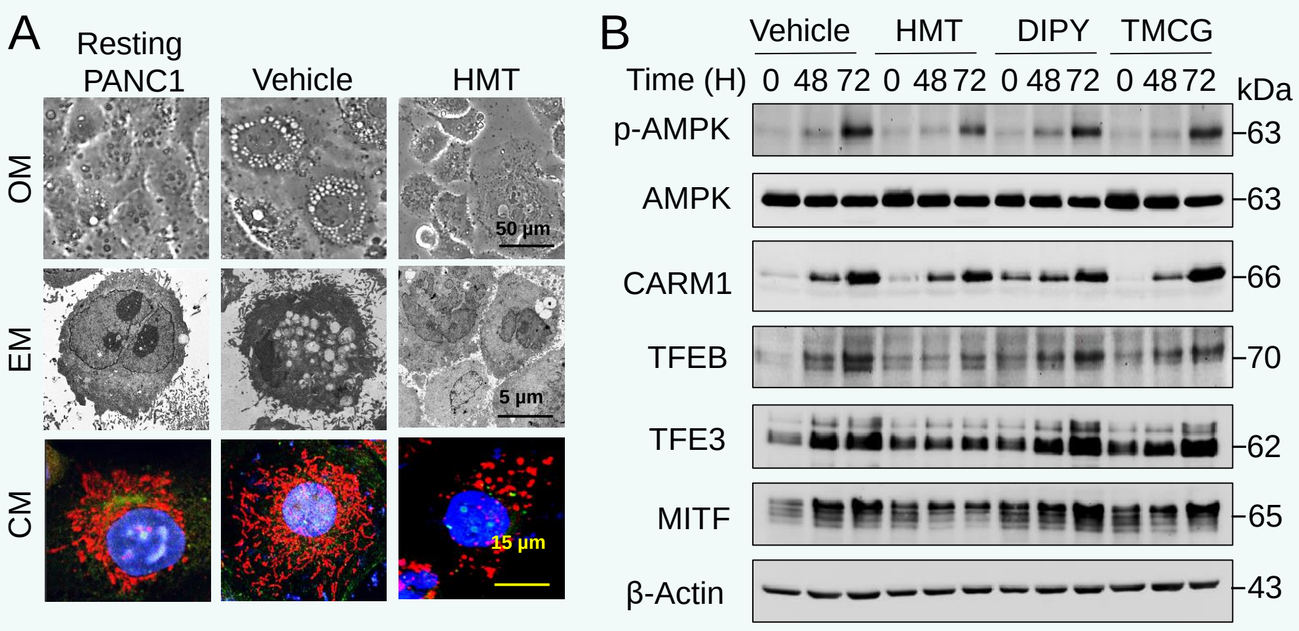
OBJECTIVE:

Autophagy is a central cellular mechanism for the elimination of damaged proteins, protein complexes, and organelles. This evolutionarily conserved process plays a crucial role in the cellular response to nutrient deprivation as well as other stresses. Many tumors become addicted to autophagy for survival, suggesting that inhibition of autophagy might be a potential broadly applicable cancer therapy.

RESULTS:

1. HMT inhibits basal autophagy in pancreatic cancer cells.



PANC1 cells under normal culture conditions underwent a time-dependent phenotypic changes compatible with autophagy and characterized by increase of round and homogeneous vesicles and evident signs of mitochondrial fusion. However, treatment of PANC1 cells with a combination of TMCG and DIPY (HMT treatment) was able to inhibit these phenotypic changes associated with basal autophagy (Fig. 1A). Inhibition of autophagy by cloroquine resulted in clear LC3-II expression/accumulation in PANC1 and HS766T, but HMT treatment inhibited early autophagy by blocking autophagosome formation (Fig. 1B). The analysis of several markers of autophagy indicated the time-dependent activation of p-AMPK and CARM1 (Fig. 1C). In addition to the activation of p-AMPK, an increase in the master regulatory genes of autophagy MITF, TFEB and TFE3 and the nuclear translocation of MITF and TFE3 was also observed. Interestingly, the treatment with HMT decreased the phosphorylation of AMPK as well as the activation of CARM1, MITF, TFEB, TFE3. and the activation of CARM1 (Fig. 1C).

Fig 1. (A) The increase in the number of vesicles in PANC1 cells under normal and HMT conditions is observed with optical microscopy (MO) and electron microscopy (EM) and mitochondrial fusion by confocal microscopy (CM). (B) Western Blot to study the level of indicated proteins in PANC1 cells at different times in control and HMT conditions. (C) Detection of LC3-II expression in two cell lines by confocal microscopy after HMT treatment. Cloroquine (CQ) was used to inhibit autophagosome formation.

2- HMT decreases the cellular methylation capacity.

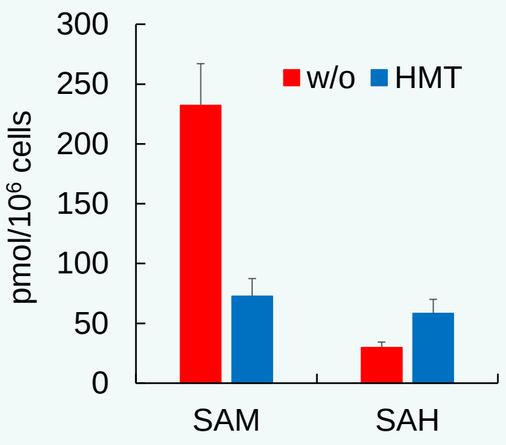
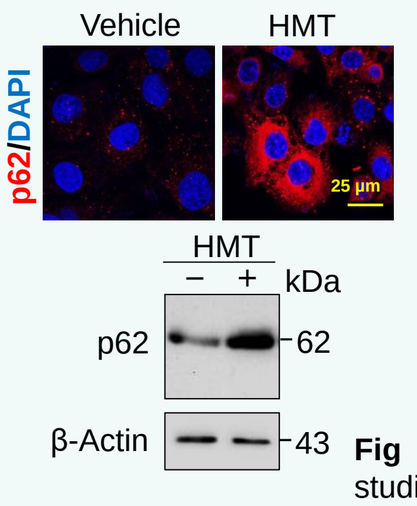


Fig 2. Histogram represent SAM/SAH ratio in PANC1 cells treated with HMT.

The ratio SAM/SAH is accepted as an indicator of cellular methylation capacity, and an increase in this ratio may predicts a higher cellular methylation status. Analysis of HMT treatment on cellular SAM/SAH ratios showed a consistent and significant shift of the SAM/SAH ratio toward a lower methylation status in cells treated with the HMT combination (Fig. 2).

3- HMT increase p62 expression in pancreatic cancer cells.



p62 protein has now been recognized as a marker for assessing autophagy. During active autophagy p62 protein levels are usually reduced but are overexpressed when autophagy is disabled. Experiments of western blot and confocal microscope images showed an increase of p62 expression after HMT treatment indicating a blockage of autophagy (Fig. 3).

Fig 3. Upregulation of p62 in response to HMT was studied by microscopy confocal and western blot assays.

4- HMT induces apoptosis in pancreatic cancer cells.

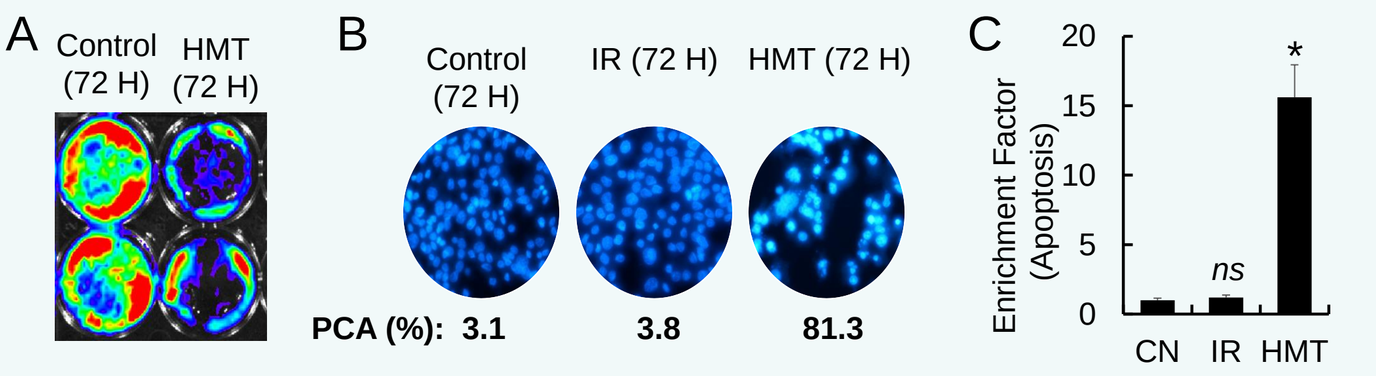


Fig 4. (A) Luciferase assays in PANC1 cells treated with HMT. (B) Hoechst stain was used to stain DNA in PANC1 cells when compared with control or ionizing radiation (IR) exposure and HMT treatment. PCA represent the percentage of apoptotic cells. (C) Histogram represent signal of apoptosis at indicated conditions by ELISA experiments.

Since HMT blocked autophagy and increased p62 labelled protein, we next checked whether HMT induced apoptosis in pancreatic cancer cells. HMT treatment of PANC1 cells resulted in less cell proliferation and cell death as observed in luciferase signal detection (Fig. 4A). To confirm this cell death and to understand whether apoptosis mechanism was activated upon HMT treatment and autophagy impairment, we performed two independent apoptosis assays (Fig. 4B and 4C). Hoechst and ELISA assays revealed an evident increase in apoptosis in HMT treated cells.

CONCLUSION:

These data indicate that pancreatic cancer cells are dependent on the autophagy process and that under nutrient limiting conditions, they can activate the necessary systems to supply cellular nutrients. However, treatment of PANC1 cells with a combination of TMCG and DIPY (HMT treatment) was able to inhibit the phenotypic changes associated with basal autophagy and to efficiently induce apoptosis.

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