Metformin Represses Autophagy in Glucose Starved Microvascular Endothelial Cells to Promote Cell Death

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Micro-vascular endothelial cells (MECs) in a growing tumour are subjected to hypoxia and nutrient/glucose starvation caused by the reduced supply and relatively high consumption rate of glucose. Metformin, the most frequently administered, anti-diabetic drug has been shown to exhibit anti-cancer activity in different types of cancer cells. Dose-dependent studies of varying concentrations (0-500µM, 1mM, 2mM, 5mM, 10mM and 20mM) of metformin on endoplasmic reticulum (ER) stress and autophagy markers were performed in the presence and absence of glucose in mouse MECs (MMECs). Results showed that 2mM metformin effectively reversed glucose starvation induced upregulation of ER stress and autophagy markers in addition to activating anti-angiogenic thrombospondin-1, while in normal glucose exposed cells higher concentrations of metformin activated ER stress. Hence we chose 2mM metformin as our working concentration for the current study. The effect of metformin on endoplasmic reticulum (ER) stress and autophagy in the glucose starved MMECs was examined in the current study. MMECs were subjected to glucose starvation for 24h and 48h in the presence and absence metformin (2mM) and the status of ER stress, autophagic, cell survival and apoptotic markers were assessed. 24h of glucose starvation significantly increased the levels of GRP78 (∼ 18 fold, n=5), ATF4 (∼ 9 fold, n=5) and CHOP (∼ 18 fold, n=5) when compared to cells exposed to 11mM glucose indicating that activation of the ER stress response is due to the accumulation of mis-folded or unfolded proteins in the ER. This was followed by activation of autophagy in glucose-starved cells as evidenced by the significant increase in the levels of LC3A-II (∼ 3 fold, n=5) and LC3B-II (∼ 5 fold, n=5), when compared to normal glucose exposed MMECs. Additionally, immunofluorescence staining for LC3B followed by confocal microscopy revealed distinct LC3B stained punctae in glucose starved MMECs compared to normal glucose exposed cells. Treatment with 2mM metformin, but not 50µM (a concentration likely achieved during the clinical use of metformin), markedly reversed the effect of glucose starvation on ER stress components as evidenced by the significant decrease in the levels of GRP78 (∼ 4 fold, n=5), ATF4 (∼ 2 fold, n=5), and CHOP (∼ 3 fold, n=5) levels when compared non-treated glucose starved MMECs. In association with the reversal of ER stress, treatment with 2mM, but not 50µM, metformin in glucose starved cells, significantly reversed the glucose starvation induced conversion of LC3A-I to LC3A-II (∼ 6 fold, n=5) and LC3B-I to LC3B-II (∼ 4 fold, n=5), and markedly reduced the formation of LC3B stained punctae, when compared to non-treated glucose starved cells, indicating a decrease in the level of autophagic activity. Gene knockdown experiments using siRNA against AMPK indicated that the effect of metformin on glucose starvation induced ER stress and autophagy are independent of AMPK. Treatment with 2mM metformin for 24h in glucose starved conditions did not show a marked change in cell viability; however, at 48h, persistent ER stress and metformin associated inhibition of autophagy promoted cell death as evidenced by the increase in the number of cells in sub G0/G1 phase of cell cycle and marked decrease in cell viability. Treatment with metformin also reduced indices of cell survival such as phosphorylation of Akt (∼ 1.9 fold, n=5) and mammalian target of rapamycin (mTOR, ∼ 1.5 fold, n=5) and inhibited downstream targets of mTOR. Our findings support the hypothesis that treatment with a high dose of metformin when used in combination with glycolytic inhibitors should inhibit pro-survival autophagy and promote cell death and potentially be of value as an effective adjunct for anti-cancer therapy.