Rapamycin promotes the survival and angiogenesis of high glucose-exposed human umbilical vein endothelial cells by improving autophagy

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A great number of diabetic complications, such as aberrant angiogenesis and sustained production of oxidative stress emerge following chronic hyperglycemia. The aim of this study was to investigate whether rapamycin, an inducer of autophagy, promotes cell survival and induces angiogenesis differentiation through inhibition of oxidative stress in human umbilical vein endothelial cells (HUVECs) under high glucose conditions. If so, does autophagy play a role in this protection? For this, HUVECS were cultured in presence of 5 mM glucose (normal) or 30 mM glucose (high glucose condition) with or without rapamycin (100 nM) for 72 hours. The possible influence of osmolarity on cell survival was excluded by the application of 30 mM of mannitol. Cell viability was measured by MTT assay. Griess method and TBARS assay were used to monitor changes in the levels of nitric oxide and malondialdehyde followed by flow cytometric analysis of intracellular ROS using DCFDA. Endothelial cells migration and angiogenic properties were assessed using scratch test and tubulogenesis assay, respectively. Formation of autophagic vacuoles was evaluated by monodansylcadaverine (MDC) staining. The expression of autophagic modulators LC3, Becline-1 and P62 was measured by using western blotting. The results indicated that high glucose concentration induced cell death and inhibited tubulogenesis that were associated with increased extra- and intracellular reactive oxygen species levels. High concentration of glucose also suppressed autophagy by reducing autophagic vacuoles formation along with accumulation of p62/SQSTM1. Rapamycin increased cell survival and significantly decreased the production of reactive oxygen species in high glucose condition and considerably promoted cell migration and tube formation. Rapamycin significantly suppressed high glucose-induced elevation of p62/SQSTM1. 3-Methyladenine, an inhibitor of autophagy, blocked the rapamycin-induced autophagy flux and protection. In summary, proangiogenic and anti-oxidative effects of rapamycin in HUVECs under high glucose condition make autophagy a promising pathway in protecting endothelial cells in diabetic induced pathologies such as foot ulcers.