

P021 Raf-1 signalling regulates pancreatic beta-cell survival and proliferation

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A reduction in pancreatic β -cell survival is an important pathogenic event in all forms of diabetes, and in the failure of clinical islet transplantation. Raf-1 kinase has been shown to play a protective role in many cell types, but its role in pancreatic β -cells is unknown. We examined whether β -cells possess Raf-1 and tested if Raf-1 function is critical for β -cell survival and proliferation. Using RT-PCR, western blot and immunofluorescence, we identified Raf-1 in primary islets and in the MIN6 β -cell line. Blocking Raf-1 activity using a specific Raf-1 inhibitor and dominant-negative Raf-1 mutants led to a reduction in β -cell proliferation detected by BrDU incorporation and increase in death, assessed using real-time imaging of propidium iodide incorporation and caspase-3 cleavage. The rapid increase in death was associated with decreased ERK phosphorylation. ERK-independent pathway downstream of Raf-1 has been proposed in other tissues and this pathway involves interactions with protein Bad. Inhibition of Raf-1 led to a striking loss of Bad phosphorylation at serine 112 and an increase in the total protein levels of Bad and Bax. Our data strongly suggest that Raf-1 signalling plays an important role regulating β -cell survival and proliferation, via both ERK-dependent and Bad-dependent mechanisms.