BCL-xL/BCL2L1 is a critical anti-apoptotic protein that promotes the survival of differentiating pancreatic cells from human pluripotent stem cells

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Abstract

The differentiation of human pluripotent stem cells into pancreatic cells involves cellular proliferation and apoptosis during cell fate transitions. However, their implications for establishing cellular identity are unclear. Here, we profiled the expression of BCL-2 family of proteins during pancreatic specification and observed an upregulation of BCL- xL, downregulation of BAK and corresponding downregulation of cleaved CASP3 representative of apoptosis. Experimental inhibition of BCL-xL reciprocally increased apoptosis and resulted in a decreased gene expression of pancreatic markers despite a compensatory increase in anti-apoptotic protein BCL-2. RNA-Seq analyses then revealed a downregulation of multiple metabolic genes upon inhibition of BCL-xL.

Follow-up bioenergetics assays revealed broad downregulation of both glycolysis and oxidative phosphorylation when BCL-xL was inhibited. Early perturbation of BCL-xL during pancreatic specification also had subsequent detrimental effects on the formation of INS^+ pancreatic beta-like cells. In conclusion, the more differentiated pancreatic progenitors are dependent on anti-apoptotic BCL-xL for survival, whereas the less differentiated pancreatic progenitors that survived after WEHI-539 treatment would exhibit a more immature phenotype.

Therefore, modulation of the expression level of BCL-xL can potentially increase the survival and robustness of pancreatic progenitors that ultimately define human pancreatic beta cell mass and function.

Figure

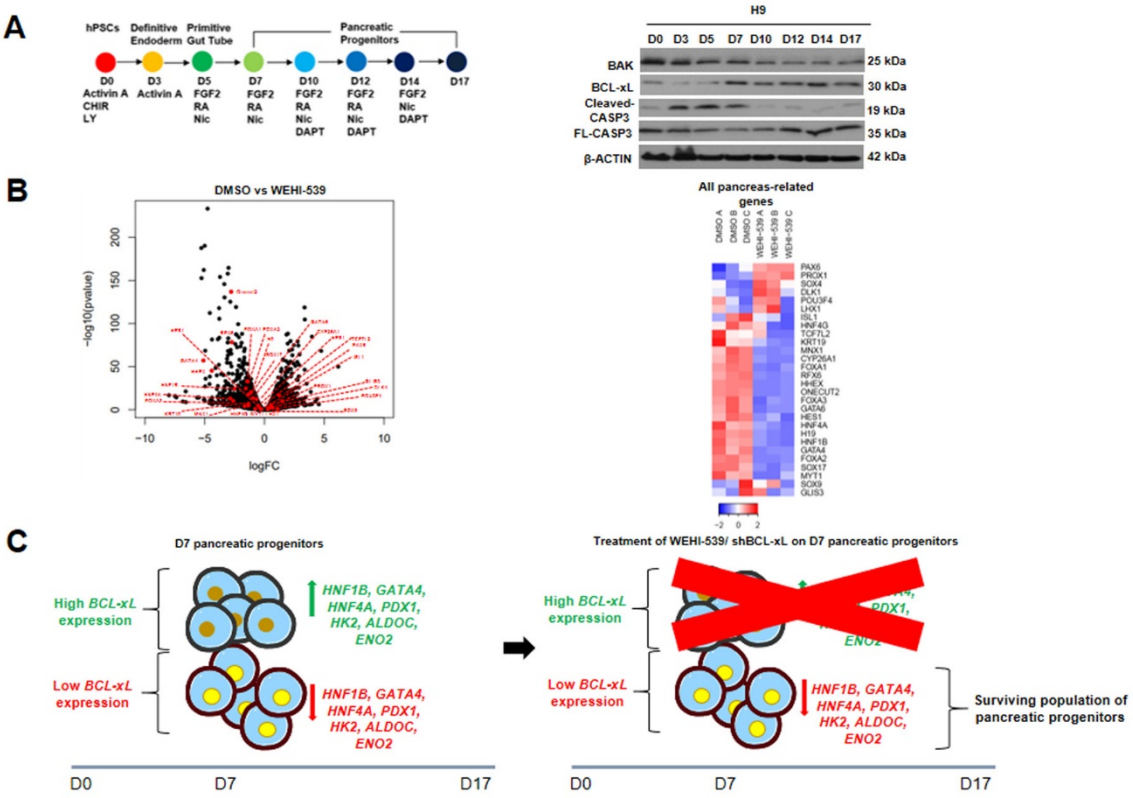

Figure Legend

(A) **Left panel:** Schematic showing 17D differentiation protocol used to generate early pancreatic progenitors. Respective growth factors used are depicted at each time point. **Right panel:** Western blot showing the expression of BCL-xL and BAK proteins over the course of 17D differentiation in H9 hESCs.

(B) Left panel: RNA-Seq analyses showing gene expression volcano plot of cells treated with DMSO or WEHI-539. **Right panel:** RNA-Seq heatmap analysis of pancreatic genes (red dots) in D7 cells treated with DMSO or WEHI-539. Colors in the heat map depict gene expression in units of SD from the mean across all samples (upregulation in red, downregulation in blue).

(C) Left panel: 2 populations of D7 pancreatic progenitors (High BCL-xL expression and low BCL-xL expression) exist during 17D differentiation. **Right panel:** Inhibition of BCL-xL leads to an indirect decrease in pancreatic and glycolytic gene expression due to the loss of more differentiated pancreatic progenitors that are more dependent on BCL-xL for survival.