Overexpression of BCL2 enhances survival of human embryonic stem cells during stress and obviates the requirement for serum factors.

**Journal:** Proc Natl Acad Sci U S A

**Publication Year:** 2011

**Authors:** Reza Ardehali, Matthew A Inlay, Shah R Ali, Chad Tang, Micha Drukker, Irving L Weissman

**PubMed link:** 21300885

**Funding Grants:** Prospective isolation of hESC-derived hematopoietic and cardiomyocyte stem cells, Stanford CIRM Training Program, Stanford CIRM Training Program

**Public Summary:**
The promise of pluripotent stem cells as a research and therapeutic tool is partly undermined by the technical challenges of generating and maintaining these cells in culture. Human embryonic stem cells (hESCs) are exquisitely sensitive to culture conditions, and require the constant presence of growth factors and other cells and structures to maintain their status as pluripotent, undifferentiated stem cells. Previous work from our laboratory demonstrated that overexpression of the prosurvival gene BCL2 in mouse embryonic stem cells led to the maintenance of mouse ESCs in culture. To determine whether this prosurvival gene could similarly protect hESCs, we generated hESC lines that express BCL2. We find that BCL2 overexpression significantly increased hESC survival and function in culture. In addition, BCL2-hESCs require basic fibroblast growth factor to remain undifferentiated. Furthermore, they maintain their pluripotency markers, form teratomas (tumors) in vivo, and differentiate into all three germ layers. Our data suggest that the BCL2 signaling pathway plays an important role in inhibiting hESC cell death, such that its overexpression in hESCs offers both a survival benefit in conditions of stress by resisting apoptosis and obviates certain requirements for culture maintenance.

**Scientific Abstract:**
The promise of pluripotent stem cells as a research and therapeutic tool is partly undermined by the technical challenges of generating and maintaining these cells in culture. Human embryonic stem cells (hESCs) are exquisitely sensitive to culture conditions, and require constant signaling by growth factors and cell-cell and cell-matrix interactions to prevent apoptosis, senescence, and differentiation. Previous work from our laboratory demonstrated that overexpression of the prosurvival gene BCL2 in mouse embryonic stem cells overrode the requirement of serum factors and feeder cells to maintain mESCs in culture. To determine whether this prosurvival gene could similarly protect hESCs, we generated hESC lines that constitutively or inducibly express BCL2. We find that BCL2 overexpression significantly decreases dissociation-induced apoptosis, resulting in enhanced colony formation from sorted single cells, and enhanced embryoid body formation. In addition, BCL2-hESCs exhibit normal growth in the absence of serum, but require basic fibroblast growth factor to remain undifferentiated. Furthermore, they maintain their pluripotency markers, form teratomas in vivo, and differentiate into all three germ layers. Our data suggest that the BCL2 signaling pathway plays an important role in inhibiting hESC apoptosis, such that its overexpression in hESCs offers both a survival benefit in conditions of stress by resisting apoptosis and obviates the requirement for serum or a feeder layer for maintenance.

**PNAS Lens Free Article Link:**

Source URL: https://www.cirm.ca.gov/about-cirm/publications/overexpression-bcl2-enhances-survival-human-embryonic-stem-cells-during