S103

Efficient Derivation of New Human Embryonic Stem Cell Lines and Elucidation of the PI3K/Akt/PKB Signal Pathway for the Self Renewal Activity of Human Embryonic Stem Cells

Sun Jong Kim

Division of Stem Cell Biology, Medical Research Center, MizMedi Hospital, Seoul 157-280

Human embryonic stem cells (hESCs), which are derived from the inner cell mass of human embryonic blastocysts, are pluripotent and able to differentiate into a broad spectrum of cell lineages. Derivations of human embryonic (hES) stem cells was first reported by Thomson et al. (1998). At present, 78 hES cell lines have been registered on the NIH registry (http://escr.nih.gov/), although only around 10 lines are available in sufficient quantity for analysis, and there is only limited data on the fundamental properties of these cell lines. There is an urgent need for more hES cell lines to be generated and characterized, as each cell line may have its own specific applications. Furthermore, the availability of more hES cell lines for comparison would aid in defining criteria for hES cells and robust methods for the growth of these cells. Up to date, unanswered questions relating to basic aspects of hES cell biology, such as the optimal conditions for deriving, characterizing, and culturing undifferentiated hES cells, make it difficult to meet the increasing demand for various well-characterized hES cell lines. To meet the increased demand for characterized hES cell lines, we established and characterized nine new lines obtained from frozen-thawed pronucleus-stage embryos. In addition, we improved the derivation efficiency from inner cell masses (to 47.4%) and optimized culture conditions for undifferentiated hES cells. After these cell lines had been maintained for over a year in vitro, they were characterized comprehensively for expression of markers of undifferentiated hES cells, karyotype, and in vitro/in vivo differentiation capacity. All of the cell lines were pluripotent, and one cell line was trisomic for chromosome 3. Improved culture techniques for hES cells should make them a good source for diverse applications in regenerative medicine, but further investigation is needed of their basic biology. In conclusion, we established and characterized nine hES cell lines. These will provide additional hES cell resources and useful preliminary data for hES cell research. In addition, identifying phenotypic differences from previously characterized hES cell lines would be valuable for defining robust criteria for hES cells. On the other hand, without physiological or environmental stimuli, they divide indefinitely in an undifferentiated state. This self-renewal is thought to result from suppression of differentiation during cell proliferation. Recent studies on hESCs demonstrated that soluble factors secreted from feeder cells are required for maintenance of the undifferentiated state and that basic fibroblast growth factor (FGF2) is essential for the undifferentiated proliferation. However, although FGF2 is generally included in the media for maintenance of hESCs, the associated mechanism of FGF2 action in these cells has not been well defined. Recent work has identified a number of signaling molecules involved in maintaining or regulating self-renewal in murine embryonic stem cells (mESCs). For example, leukemia inhibitory factor (LIF)-induced STAT3/ERK signals were shown to determine the fate of proliferating undifferentiated ES cells, and activation of Src family tyrosine kinases or Wnt signaling was found to be important for maintaining self-renewal of mESCs. Phosphatidylinositol 3-kinase (PI3K)-dependent signaling was also shown to promote mESC survival and proliferation. Additionally, extracellular matrix (ECM) molecules were shown to induce signals for promoting proliferation and long-term maintenance of undifferentiated hESCs. However, the precise signaling mechanism underlying self-renewal of hESCs is poorly understood. In this study, we determined the roles of FGF2 in maintaining hESC self-renewal. Withdrawal of FGF2 from the media led to acquisition of typical differentiated characteristics in hESCs. In the presence of FGF2, which is normally required for proliferation in an undifferentiated state, inhibition of PI3K/Akt/PKB signal stimulated differentiation and attenuated the expression of extracellular matrix (ECM) molecules such as collagen type IV 1, laminin 1, and laminin receptor. These ECM are key factors in sustaining the pluripotency of hESC characteristics. We suggest that FGF2 maintains hESC self-renewal by supporting stable expression of ECM molecules through activation of the PI3K/Akt/PKB pathway. In conclusion, the present study is the first to elucidate why FGF2 should be included in the media in a long-term hESC culture, and that normal regulation of ECM molecules by FGF2-dependent activation of PI3K/Akt/PKB signaling contributes to the self-renewal maintenance of hESCs. Considering their potential for cell therapy against many human diseases, the insights on the molecular mechanisms underlying the self-renewal and differentiation of hESCs are significant. Moreover, we expect that our study will provide a new understanding of the developmental signals that regulate early human embryogenesis.