Alternative methods for culturing human embryonic stem cells

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Introduction: The availability of human embryonic stem (hES) cells reflects their outstanding potential for research areas such as human developmental biology, teratology and cell-based therapies. To allow their continuous growth in an undifferentiated state, isolation and culturing were traditionally conducted on mouse embryonic fibroblast (MEF) feeder layers, using medium supplemented with fetal bovine serum (FBS). However, these conditions allow the possible exposure of the cells to animal pathogens. Since both research and future clinical application require an animal-free and well defined culture system for hES cells, these conditions would prevent the use of hES cells in human therapy.
This study confronts this challenge and describes optional culture conditions based on medium supplemented with serum replacement (SR).

Methods: HES cell lines I3, I6, I3.2 and H9 were transferred from the MEF culture system to medium supplemented with 15% SR and 4ng/ml bFGF on either MEF matrix, foreskin fibroblast matrix, Matrigel matrix or fibronectin. When fresh (and unconditioned) medium was used, additional known growth factors were added. Following a prolonged culture of more than 20 passages, cells were examined for ES cell characteristics.

Results: All hES cell lines maintained their ES cell features: They continued to proliferate as undifferentiated cells for at least 20 passages as demonstrated by the expression of undifferentiated-specific surface markers; When grown in suspension for over 17 passages, they formed embryoid bodies similar to those formed on MEFs; *In vivo*, the cells created teratomas containing representative tissues of the three embryonic germ layers, and normal karyotypes were retained. All matrices were found to support hES cell culture with the same efficiency.

Conclusions: The culture system presented here has two major advantages: (1) elimination or significant restriction of hES cells' exposure to animal pathogens, and (2) application of a well-defined culture system.