Title	Study of extracellular matrix for the culture of human embryonic stem cells
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opment of a defined ECM and culture media that allow the propagation of undifferentiated, genetically and epigenetically stable hESC is essential for the future therapeutic application of hESC and induced pluripotent stem cells.

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STUDY OF EXTRACELLULAR MATRIX FOR THE CULTURE OF HUMAN EMBRYONIC STEM CELLS

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Background: The development of clinical grade human embryonic stem cells (hESC) suitable for cell therapy requires defined components for hESC culture. We hypothesize that the feeder cells used for hESC culture produce extracellular matrix molecules supporting the growth of hESC. Materials and Methods: The profiles of mRNA and extracellular matrix production of two human feeder cell lines, human foreskin fibroblast (hFF-1) and human lung fibroblast (WI-38) were correlated with their ability to maintain the pluripotency of a hESC line, BG01V. The mRNA expression profile and matrix production were determined by Affymetrix microarray chip and liquid chromatography/mass spectrometry/mass spectrometry analysis, respectively. Results: Using a morphological grading system, hFF-1 but not WI-38 supported the pluripotency of BG01V. The observation was supported by over-expression of early differentiation markers KRT-8 and -18 mRNA in BG01V cells cultured on WI-38, though the mRNA expression of pluripotent markers Nanog, Oct4 and TRA-1-81 in BG01V cultured with hFF-1 and WI-38 was similar. Microarray analysis showed that the expression of 530 transcripts was 2-fold higher ($P<0.05$) in hFF-1 than in WI-38. The microarray data were validated by real-time PCR on 12 differentially expressed genes related to secretory and extracellular matrix molecules. Proteomic profiling of the extracellular matrix molecules also demonstrated differences between the two feeder cell lines. Both microarray and proteomic analysis identified sulfatase 1 (SUFL1) and chemokine (C-X-C Motif) ligand 12 (CXCL12) were higher in hFF-1. Conclusion: The difference in the ability of feeder cells in maintaining the pleuripotency of hESC may be related to the differential secretion of matrix molecules by the feeder cells.

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CELL-SUBSTRATE INTERACTIONS AND HUMAN EMBRYONIC STEM CELL PLURIPOTENCY

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Human embryonic stem (hES) cells typically have a distinctive morphology, with a high nuclear:cytoplasmic ratio and tight epithelial-like, cell-cell contacts. However they also have a requirement for substrate-bound growth, commonly either on feeder cells or an extracellular matrix (ECM) substrate. We have therefore investigated the role of cell-matrix interactions in maintaining hES cell pluripotency. Immunostaining showed that pluripotent hES cells cultured on fibronectin exhibited high levels of active β1 integrin. Surprisingly, this was predominantly located at the cell periphery at sites of cell-cell contact where fibronectin was also found. To understand how hES cell-ECM interactions might effect pluripotency, we sought to manipulate hES cell-substrate interactions and cytoarchitecture. Firstly, we investigated whether the inhibition of β1 integrin by blocking antibodies would affect hES cell morphology and influence pluripotency. We found that blocking anti-β1 inhibited cell adhesion to fibronectin but not cell-cell interaction. It induced rapid hES cell differentiation as demonstrated by decreased Nanog expression. Pluripotent hES cells exhibited a cortical actin cytoskeleton with few if any stress fibres and focal adhesions. Immunostaining for focal adhesion components paxillin and vinculin showed high levels of cytoplasmic staining. Despite the lack of focal adhesions, we found activated focal adhesion kinase in pluripotent stem cells and high levels of active RhoA involved in focal adhesion formation and signalling. As hES cells began to differentiate their cell shape changed to a spread morphology, with looser cell-cell contacts and more cell-substrate interaction. Immunostaining showed active β1 integrin and paxillin and vinculin, now localised in focal adhesions apparently in contact with actin stress fibres. Despite the clear change in localisation and cytoskeleton we found protein levels of β1 integrin, paxillin and vinculin remained unchanged upon differentiation. Differentiated cells exhibited lower levels of active RhoA. The inhibition of ROCK, a downstream effector of RhoA, has been reported to enhance the survival of human embryonic stem cells. We found that ROCK inhibitor enhanced hES cell clonogenicity on fibronectin in the absence of serum. Since manipulation of cell shape via RhoA activity can influence mesenchymal stem cell differentiation we investigated its inhibition in hES cells. We found that inhibition of RhoA by C3 transferase lead to dispersion of E-cadherin junctions in hES cell colonies on fibronectin but Nanog expression remained unchanged. Interestingly, despite the high levels of active RhoA we found the addition of lysophosphatidic acid, commonly used to activate RhoA and thus induce focal adhesion formation, led to hES cell differentiation: hESC lost Nanog expression with the simultaneous formation of focal adhesions and actin stress fibres. We are now investigating how RhoA and β1 integrin signalling interact and how these signalling pathways might affect other pathways known to influence hES cell pluripotency.

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INTEGRINS EXPRESSION PROFILE IN HUMAN ES AND IPS CELLS IN THE DEFINED CULTURE CONDITIONS

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Human ES (hES) and iPS cells are expected to be useful tools for drug screening such as toxicity evaluation. It is known that characteristics of hES and iPS cells are different among each cell line. There is a need to develop effective evaluation method and perform characterization of the cells prior to applying in the drug screening; however, it is difficult with the conventional culture method using feeder cells. Thus, we previously developed a simple, defined, serum-free culture medium (hESF9 for human, ESF7 for mouse). In the previous study, our results revealed that extracellular matrix (ECM) components affect self-renewal of mouse embryonic stem cells (mESCs) in ESF7. The ECM signaling is strongly affected by integrins, which is a large family of cell surface adhesion receptors, and the downstream signal is involved in differentiation and proliferation of the cell. mESCs cultured in ESF7 with each ECM components as culture substrata expressed integrin receptor complex for fibronectin and laminin, and their integrin expression is upregulated by differentiation process. A similar interaction between integrins and ECM in hESCs is inferred from the results of mESCs. Profiling of the integrin expression by examining the differences in the appearance of integrins and ECM in each cell lines of hES and iPS cells can establish effective evaluation method and characterization of the cell source. We perform attachment assay of the cell (hES;KhES-1 and KhES-3 human iPS; dotcom, squeaky and Tic cell lines) in hESF9, and examine the influences of ECM to the cell proliferation and the pluripotency of the cells. Also the expression levels of integrin subunits on