GENE PROFILING AND PATTERN FORMATION OF EMBRYONIC STEM CELLS: CULTURE OF RHESUS EMBRYONIC STEM CELLS ON FEEDER CELLS VS. CELL-FREE LAMININ

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Recent reports on feeder-free growth of undifferentiated human embryonic stem cells (ESCs) (*Xu et al., Nat. Biotech. 19, 971-974, 2001*) have prompted our interest in possible influences that such differing culture conditions may have on the maintenance of the phenotype of ESCs. The (pluripotent) undifferentiated stem cell state is typically associated with the expression of characteristic markers like stage specific embryonic antigen-3 (SSEA-3), SSEA-4, tumor rejection antigen-1-60 (TRA-1-60), TRA-1-81, the enzyme alkaline phosphatase and the transcription factor Oct-4. Here, we have analyzed the expression of these markers in correlation with the morphology of primate ESCs, i.e. Rhesus monkey embryonic stem cells (rESCs) (R366.4, WiCell Research Institute, Madinson, WI, USA) cultured for 2-4 days on either (i) mouse embryonic fibroblasts (MEFs) or (ii) laminin-coated coverslips (LAMs) in MEF-conditioned medium.

When maintained on MEFs (i), the rESCs aggregated and formed distinct colonies. Examination of these colonies by TEM / REM demonstrated a multilayered arrangement of cells and a broad variation in cell morphology underlining the importance of molecular criteria for stem cell identification. Within single colonies, many of the cells expressed SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase but not SSEA-1. The percentage of cells expressing these markers was determined by immunofluorescence microscopy indicating cells that had retained a stem cell phenotype. Oct-4 expression was detectable within cultures by Northern blot analysis.

When maintained on LAMs (ii), the rESCs grew as a confluent monolayer rather than forming multilayered colonies. Only a small proportion of cells continued to express a stem cell-like morphology. Similar to cultures on feeders, many of the rESCs down-regulated their set of stem cell markers expressed. Oct-4 was still detected within these cell cultures although the percentage of positive cells was not determined in this case.

In summary, primate ESCs (R366.4 cells) were found to become phenotypically heterogenous under both conditions tested, i.e. on either

mouse embryonic fibroblasts or laminin-coated coverslips. Many cells down-regulated markers for undifferentiated embryonic cells while actively proliferating stem cells persisted and kept cultures going. Further studies are needed in order to clarify the obviously complex population dynamics in these cultures and to relate this to concepts concerning stem cell niches and plasticity as well as to the pattern formation potential of ESCs.

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