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[Abstract 25]

Rhesus embryonic stem cell culture on feeder cells vs. cell-free laminin

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Primate embryonic stem cells are ususally grown on mouse embryonic feeder cells (MEF) in order to keep them in an undifferentiated state since they do not response to LIF. Xu et al. (Nat. Biotech. 19, 971-974, 2001) recently reported on cultivation of human embryonic stem cells feeder-free on laminin or matrigel matrices in combination with MEF-conditioned medium.
We adopted this system to Rhesus embryonic stem cells (rhESC) and established a feeder-free system on laminin plus MEF-conditioned medium for more than 30 passages and compared it with rhESC grown in the standard system on MEF.
When maintained on MEF, rhESC aggregated and formed distinct colonies. In the TEM or REM, these colonies appeared multilayered and showed a broad variation in cell morphology with columnar epithelial cells lying next to cells that morphologically appeared undifferentiated. Immunohistochemically, many cells expressed the ES markers stage-specific embryonic antigens (SSEA)-3 and SSEA-4 and tumor-rejection antigens (TRA)-1-60 and TRA-1-81, as well as alkaline phosphatase (AP). Nevertheless, the colonies were always mosaic with cells expressing epithelial markers ZO-1, cytokeratin, or vimentin. Oct-4 expression remained detectable by Northern blot analysis.
When maintained on laminin, the rhESC tended to grow as confluent monolayers with predominantly fibroblastic morphology. AP, SSEA-3 and SSEA-4 as well as TRA1-60 and TRA-1-81 seemed to be mildly downregulated in these cultures. Similar to standard MEF-cultures they expressed epithelial as well as fibroblast markers. Nevertheless, the Oct-4 espression was maintained indicating that within the heterogeneous colonies at least a small portion of cells retained their embryonic stem cell character.
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.