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Studies of Culture and Differentiation of Primordial Germ Cells in Rats

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Our study focuses on morphology of several kinds of primodial germ cells (PGCs)-derived cells in absence of mouse fibroblast feeder layer using light and electron microscopy in order to enrich evidence of pluripotentialty of PGCs. Gonadal ridges of rat embryos were disaggregated. One group was cultured on a mouse fibroblast feeder layer, the other was cultured without any feeder layer. Both cultures were grown in DMEM with hrLIF and hrbFGF. Cells were fixed for detection of ALP activity and were observed under the electron microscope. The results showed that PGCs gave rise to singly large and round cells contains mitochondria and ribosomes in the presence of mouse fibroblast feeder layer, hrbFGF and hrLIF. PGCs were positive to ALP reaction. This suggested that PGCs be in undifferentiated state. While in absence of feeder layer though in presence of hrLIF and hrbFGF PGCs varied in shape and often with pseudopodia and then differentiated into cardiomyocytes, neuron-like cells, secretory cells, epidermic cells and so on. These results suggested that PGCs have pluripotentialty to differentiate *in vitro* into derivatives of the three embryonic germ layers, and PGCs be undifferentiated inhibited by feeder layer.

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Preliminary Culture of Adult Human Mesenchymal Stem Cells

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Human mesenchymal stem cells (hMSCs) which are present in adult marrow, can replicate as undifferentiated to lineages of mesenchymal tissue, therefore they are thought to be multipotent cells. Although bone marrow is the major source of adult hematopoietic stem cells (HSCs), there is also a few MSCs. We isolated and cultured cells that have the characteristics of human mesenchymal stem cell from marrow aspirates. Marrow aspirate was centrifuged. The cell pellets were loaded onto Percoll and spun. Then chose the low density cells containing mesenchymal stem cell to culture routinely. Human MSCs remained an attached well-spread morphology as a monolayer in vitro. Almost cells were fibroblastic, with a few adipocytic, polygonal or round. The cells did not differentiate spontaneously during culture expansion. Histochemical staining for alkaline phosphatase (AP) yielded positive. It suggested that MSCs were undifferentiated to a certain extent. The MSCs that appeared to be negative for CD34 surface antigen were different from marrow hematopoietic stem cells that remained positive for CD34. At passage 3, cultured marrow MSCs maintained a normal karyotype.