عنوان مقاله:

Isolation and primary culture of ES-like colonies from NMRI mouse embryos

محل انتشار:

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نویسندگان:

Seyed Hassan Eftekhar Vaghefi - *Department of Anatomy, Afzalipour School of Medicine, Kerman, Iran Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran*

Nehleh Zareii Fard - *Department of Anatomy, School of Medicine, Hormozgan University of Medical Sciences, Hormozgan, Iran.*

Zhinoososadat Shahidzadeh - *Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.*

Seyed Noureddin Nematollahi-mahani - *Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran. Afzal Research Institute (NGO), Kerman, Iran.*

خلاصه مقاله:

Background: Embryonic stem (ES) cells are pluripotent cells conventionally isolated from early embryos. Studies have shown that ES cells serve as a practical model for biomedical studies.Objective: The aim of the present study was to optimize culture conditions for establishment of ES-like colonies from NMRI mouse blastocysts as well as 2-cell stage embryos.Materials and Methods: Both expanded blastocysts and 2-cell stage embryos were co-cultured on mouse embryonic fibroblast (MEF). Plating capacity and formation of Inner cell mass (ICM) were examined daily. The differentiation and growth behavior of ICM cells were examined with various procedures. ICMs derived from initially cultured 2-cell or blastocyst embryos were disaggregated either mechanically or enzymatically, and seeded onto MEF with or without leukemia inhibitory factor (LIF). The resulted colonies were disaggregated and reseeded onto MEF and the colonies that were morphologically similar to ES cells were evaluated for pluripotency using alkaline phosphatase (ALP) expression as a stem cell marker.Results: No morphologically good ES-like colony was isolated from 2-cell embryos after passages, while 273 (79%) good-looking ICMs were isolated from 352 blastocysts. Four sets of colonies remained undifferentiated following passages. Enzymatic method of ICM disaggregation was superior to the mechanical method. Besides, all ES-like colonies were obtained from the ICMs cultured in presence of MEF and LIF.Conclusion: Our results show that NMRI mouse ICMs could be isolated and cultured from blastocyst stage embryos with a suitable culture system and ES-like cell colonies remain undifferentiated when cultured with MEF and LIF.

کلمات کلیدی:

NMRI mouse, Inner cell mass, Embryonic stem cell