

研究报告

完整的供体细胞膜及供体胞质对小鼠体细胞克隆胚发育的影响

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摘要

利用显微注射和电融合的方法都可以成功地获得体细胞克隆小鼠, 由于电融合法操作耗时, 融合率低, 因而大多数克隆小鼠是采用注射方法。而注射法需要将供体细胞核从细胞中分离出来, 此分离操作有可能导致对DNA的损伤, 曾有人使用直径较粗的注射管进行完整的供体细胞注射, 这种方法操作相对简单而且对供体核没有损伤。为了研究这种方法在小鼠核移植中是否适用, 本实验使用完整的小鼠卵丘细胞作供体, 进行显微注射, 结果显示, 完整的卵丘细胞注入卵母细胞后, 无论在1小时或者6小时激活, 大部分的重构胚在2细胞期碎裂, 而去掉细胞膜的供体体细胞核注入卵母细胞后, 重构胚可以卵裂并进一步发育。卵母细胞去核后不注射供体也发生碎裂, 大部分的孤雌胚(不去核)在完整的卵丘细胞被注入后同样发生碎裂。在供体卵丘细胞刚破膜后即被注入卵胞质和供核被充分剥离后注入两种情况下获得的重构胚的体外发育中, 前者发育各期的比率显著低于后者。这些结果说明完整的卵丘细胞膜阻碍了卵胞质对体细胞核的重编程作用, 造成碎裂; 注入卵胞质的供体质膜和胞质成分影响了克隆胚的体外发育。

关键词 [核移植](#) [小鼠](#) [胚胎发育](#) [卵丘细胞](#) [细胞质](#)

分类号

Effect of intact donor cell membrane and cytoplasm of cumulus on development of mouse somatic clones *in vitro*

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Abstract

<P>Reconstructed embryos can be got by two nuclear transfer methods, fusion and microinjection. However, the fusion method is time-taking and has low efficiency. Microinjection involved the donor nucleus isolation, which may lead to the damage of the nucleus DNA. Cloning efficiency may be reduced by low fusion rate of the cell fusion method and damaged DNA of microinjection method. So some researchers used the intracytoplasmic injection of the whole-cell donor, which was simple and less labor-intensive for cloning study. Here we used the intact cumulus cell as donor to study the feasibility of the microinjection method in mouse nuclear transfer. After the whole cell injection of the cumulus, most of the couplets were fragmented following activation at 1 or 6 h after injection. Whereas those only the nucleus after isolation from the cumulus was injected cleaved and developed normally. The control group of oocytes, which were not injected with the donor, was also fragmented after enucleation. Most of the couplets of the parthenotes after intact cumulus cell injection were fragmented. The group of those in which the donor was just broken and then injected (nucleus with

most of the broken membrane and the cytoplasm were injected) developed as a lower rate compared to those only isolated nucleus being injected. These results suggest that the intact donor membrane of cumulus cell hinder the re-programming of oocyte for somatic nucleus and components of donor cytoplasm and membrane affected the devel-opment of the clones in vitro.
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