Mitomycin C cause chromosome change of human embryonic stem cells

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How to maintain the genetic integrity of cultured human embryonic stem cells (hESCs) is raising crucial concerns to future clinical use in regenerative medicine. At present, application of mitotic-inactivated mouse embryonic fibroblasts (MEFs) supplemented with 4 ng/ml bFGF is the routine culture system to maintain the undifferentiated state of hESCs. Mitomycin C, a DNA damage agent, is usually used for preparation of feeder cells in many laboratories. However, to what extent mitomycin C affects the karyotypic stability of hESCs is not clear. In this study, hESCs were treated by different concentrations of mitomycin C (1~0.001 µg/ml) both in feedercontaining and feeder-free system for 24 or 48 h, and karyotype analysis was performed thereafter. Results showed that 1 µg/ml mitomycin C was lethal to hESCs for 48 h and during the first 24 h hESCs differentiated in large numbers, no metaphase found either after 24 or 48 h. In 0.1 and 0.01 µg/ml concentrations, chromosome changes were found both after 24 and 48 h. The abnormalities included breakage and deletion. However, hESCs were normal in 0.001 µg/ml and untreated concentration. Although hESCs did not change in 0.001 µg/ml (1 ng/ml) concentration, we did not know whether long-term culture of hESCs in low concentration of mitomycin C would have any effects. So we further cultured hESCs in 1~0.02 ng/ml for 10 passages. Results showed that in feeder-containing systems (both mouse and human feeder cells) karyotypes were found changed even in 0.02 ng/ml concentration, but remained normal in feeder free system in the same concentration. In other 3 concentrations: 1 ng/ml, 0.3 ng/ml and 0.1 ng/ml, hESCs karyotypes were changed with unknown derivative chromosomes found. We did not find any chromosome abnormalities in untreated group. In a word, our results suggested that mitomycin C could cause the karyotypic changes of hESCs, which might be a potential risk for clinical application.

Keywords: mitomycin C, hESCs, karyotype, abnormality

Cell Research (2008) 18:s124. doi: 10.1038/cr.2008.214; published online 4 August 2008

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