Differential Distribution of Ganglioside GM3 during Apoptosis in Preimplantation Mouse Embryos

Eun-Jin Ju¹, Dong-Hoon Kwak¹, Dae-Hoon Lee¹, Sung-Min Kim¹, Kyu-Yong Jung², Seo-Ul Lee², Su-Il Do³, Yong-Il Park⁴ and Young-Kug Choo¹

¹Department of Biological Science, College of Natural Sciences, Wonkwang University, Jeonbuk 570-749, Korea ²Department of Pharmacology, Wonkwang University School of Medicine, Jeonbuk 570-749, Korea

³Ajou University, Department of Life Science, San 5, Wonchon-dong, Suwon 443-749, Korea

^⁴Department of Biotechnology, Catholic University, Kyanggi-do, 420-749, Korea

Apoptosis is an essential physiologic process used in almost all tissues to remove damaged or superfluous cells. Apoptosis may occur in early embryos in which the execution of essential developmental events has failed. Gangliosides, sialyated glycosphingolipids, are expressed in the CNS and have been proposed to regulate cell growth and differentiation. Here, we inverstigated the expressional patterns of ganglioside GM3 in apoptotic cell. Mouse preimplantation embryos isolated through in vitro fertilization. In our experiments three apoptosis inducers (actinomycin D, camptothecin and cycloheximide) were used in an culture medium for 15h at the 4-cell stage (day 2) of mouse embryos, followed by further development in a pure culture medium until fixation on day 3, 4 and 5. Apoptotic cell was indicated using the terminal deoxynucleotidyl transferase (TdT) nick-end labelling (TUNEL). Three inductors of apoptosis significantly increased the percentage of apoptotic cell and reduced total cell counts. The induced embryos did not reach the blastocyst stage. The number of blastomeres was decreased, and staining with Hoechst 33342 revealed a significant percentage of apoptotic nuclei. Expressional patterns of GM3 in apoptotic cell was greatly different from that in control embryos. GM3

synthase mRNA was expressed in apoptosis induced embryos using RT-PCR. These results suggest that GM3, a simple ganglioside, may regulate cell proliferation and death in embryos.

Key words) Apoptosis, Ganglioside GM3, TUNEL, Immunofluorescense stain