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Establishment and Aging Process Analysis in Dermal Fibroblast Cell Culture of Green Turtle (Chelonia mydas)

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Abstract : Green turtle (Chelonia mydas) is one of well known long-lived turtle. Its age can reach 100 years old. Senescence in green turtle is an interesting process to study because until now no clear explanation has been established about senescence at cellular or molecular level in this species. Since 1999, green turtle announced as an endangered species. Hence, establishment of fibroblast skin cell culture of green turtle may be material for future study of senescence. One common marker used for detecting senescence is telomere shortening. Reduced telomerase activity, the reverse transcriptase enzyme which adds TTAGGG DNA sequence to telomere end, may also cause senescence. The purpose of this research are establish and identify green turtle fibroblast skin cell culture and also compare telomere length and telomerase activity from passage 5 and 14. Primary cell culture made with primary explant method then cultured in Leibovitz-15 (Sigma) supplemented by 10% Fetal Bovine Serum (Sigma) and 100 U/mL Penicillin/Streptomycin (Sigma) at 30 ± 1oC. Cells identified with Rabbit Anti-Vimentin Polyclonal Antibody (Abcam) and Goat Polyclonal Antibody (Abcam) using confocal microscope (Zeiss LSM 170). Telomere length obtained using TeloTAGGG Telomere Length Assay (Roche) while telomerase activity obtained using TeloTAGGG Telomerase PCR ElisaPlus (Roche). Primary cell culture from green turtle skin had fibroblastic morphology and immunocytochemistry test with vimentin antibody proved the culture was fibroblast cell. Measurement of telomere length and telomerase activity showed that telomere length and telomerase activity of passage 14 was greater than passage 5. However, based on morphology, green turtle fibroblast skin cell culture showed senescent morphology. Based on the analysis of telomere length and telomerase activity, suspected fibroblast skin cell culture of green turtles is not undergo aging through telomere shortening.

Keywords : cell culture, chelonia mydas, telomerase, telomere, senescence
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