Abstract

Development of venom gland cells from Trimeresurus gramineus (Taiwan green habu) to produce venom is important as an additional source to manufacture antivenom and as a source of useful biologically active molecules to become a novel therapeutic agents, diagnosis and research tool. We established the immunohistochemical method to identify venom gland cells and in best result, ten thousand epithelial cells from venom gland obtained by magnetic adsorption method. In our studies, venom gland cell could not attached on culture plate which were precoated with calf skin collagen, rhodostomin or snake skin matrix proteins under 100 Kd. Immunocytochemical staining of primary venom gland cell, 10 weeks in culture after being placed in immunflouresence slide, using pan-keratin antibodies to recognize epithelial cell venom gland cell are stained positive. After 1, 3 and 10 days in culture, they were stained positively by DAPI. The cultures of venom gland cells can be maintained up to 120 days and 4 passages. The venom gland cells were stimulated with EGF or ACh and total protein in culture medium did not change significantly. Cells could not proliferate even by large T antigen transfection. Albumin depletion kit depleted 96.1% protein of Trimeresurus gramineus venom, 93.4% protein of bovine serum albumin and 95.2% protein of cell supernatant. The secretion of venom could not be demonstrated in supernatant of 14-day gland cell culture by SDS-PAGE and Western blotting. A rapid and sensitive ELISA for detecting Trimeresurus gramineus venom was uccessfully established. The optimal concentration of anti-Trimeresurus gramineus and Trimeresurus mucosquamatus serum was between 10mg/ml to 0.1mg/ml. This ELISA test can detect venom with the lower detection limit of 1ng/ml.

Keywords：T. gramineus；snake venom；venom gland cell；anti-venom serum