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285. Generation of Immortalized Neuronal Cell Lines Derived from Bovine Hypothalamic Arcuate Nucleus and Medial Preoptic Area.

Fuko Matsuda[1], Fuko Matsuda[1], Yuta Suetomi[1], Ahmed S. Hassaneen[1], Masahiro Kato[1], Shuichi Matsuyama[2], Koji Kimura[2], Satoshi Ohkura[1]. [1]Nagoya University, Nagoya, Aichi, Japan; [2]NARO Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, Japan

Neurons essential for reproductive regulation, such as GnRH and kisspeptin neurons, are mainly located in the hypothalamic arcuate nucleus (ARC) or medial preoptic area (mPOA) in mammals. However, the precise functions of these neurons are not fully examined at a cellular level, especially in domestic animals including cattle. Therefore, hypothalamic neurosecretory cell lines for analyzing cellular mechanisms that control domestic animal reproduction are required. In this study, we tried to generate neuronal cell lines derived from either the ARC or mPOA of cattle. A hypothalamus was obtained from a 6-month-old Japanese Black steer. The brain was obtained from the steer under deep anesthesia, and then the ARC and mPOA tissues were dissected out. Each tissue was dispersed using papain and cultured on poly-L-lysine-coated plates with Neurobasal-A medium containing fetal bovine serum, B-27 supplement, L-glutamine and basic fibroblast growth factor. After 24 hours from the start of culture, cells were washed with phosphate buffered saline and added new medium and cultured for additional 24 hours. Then lentiviral vector containing SV40 large T antigen and neomycin resistance gene was added to the primary cultured hypothalamic cells. Seventy-two hours after the infection, culture medium was changed and the next day G418 was added to select cells in which the lentiviral genes were inserted. After the 2 weeks selection by G418, we performed cell cloning of the obtained cell population. Next, we examined gene expressions of the cell clones by RT-PCR. Total RNA was extracted and then cDNA was synthesized from each cell clone, and expression of neuronal (neuron specific enolase, NSE) or glial (glial fibrillary acidic protein, GFAP) markers was evaluated by PCR. From the expression pattern of these markers, we determined neuron-derived cell clones. Subsets of the bovine ARC and mPOA tissues were also provided for RT-PCR analyses as positive controls for NSE and GFAP expressions. After the infection of lentivirus, primary cultured bovine hypothalamic cells acquired strong proliferative activity, suggesting that these cells were immortalized by insertion of SV40 large T antigen. We obtained more than 50 cell clones from both ARC- and mPOA-derived immortalized cell populations. By RT-PCR analyses, we confirmed that both NSE and GFAP were expressed in bovine ARC and mPOA tissues. As for immortalized cell clones, we found NSE-positive and GFAP-negative cell clones, which were defined as neuron-derived cell lines. In summary, we succeeded to obtain a number of neuronal cell lines derived from bovine ARC and mPOA. Further expressional analysis including neuropeptides such as GnRH, kisspeptin, neurokinin B and dynorphin A will determine neuronal cell lines appropriate for examining central mechanism regulating ruminant reproduction in vitro. This work was supported in part by the Research Program on Innovative Technologies for Animal Breeding, Reproduction, and Vaccine Development form the Agriculture, Forestry and Fisheries Research Council, Japan.

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