Isolation of Neurosphere-Like Bodies from Adult Human Amygdala


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Abstract
In the last century, neural stem cells are used in a lot of studies for basic and therapeutic investigations. Several sources are identified for neural stem cells including embryonic, fetal and adult stem cells. Although most of studies have focused in embryonic as well as fetal cells due to their capacity to generate progenies, these cells have some problems such as immunological, availability and ethical concerns. Among adult sources for neural stem cells, two areas in adult mammalian brain including the subventricular zone of lateral wall of lateral ventricle and the subgranular zone of hippocampus are identified as niches with neural stem/progenitor cells (NS/PCs). According to previous studies, other adult mammalian brain regions may have the quiescent cells which generate stem-like cells in vitro. To date, a few researches have addressed the isolation of NS/PCs from adult human amygdala. The aim of the present study was to evaluate the ability of human amygdala tissue to generate neurosheres. The amygdala specimens were obtained from five patients suffering from refractory temporal lobe epilepsy and subjected to amigdalo-hippocampectomy. After removing the pia mater and associated blood vessels, the tissue was dissociated enzymatically. Then, the single cells were cultured in neurosphere medium containing 20 ng/ml Fibroblast growth factor, 20 ng/ml epidermal growth factor, B27 supplement and N2 supplement in non-coated flasks. Growth factors were added twice a week. Additional neurosphere medium was administered once every week. The efficacy and number of spheres and cells were evaluated. Four days after primary culture of amygdala tissue, small free floating spheres were appeared. The proliferation of the cells slowly continued to day 15 at which passage was done and neurospheres dissociate into single cells. The number of spheres and cells increased after each passage. Here, we showed for the first time in Iran the possibility of isolating proliferating neurospheres from patients with refractory epilepsy during interventional surgery.

Keywords: Amygdala, Human, Temporal Lobe Epilepsy, Neurosphere.

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