Successful in vitro expansion and Characterization of Human Enteric Neuronal cells- A step towards Cell based therapies for Hirschsprung’s disease

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BACKGROUND

The Enteric Nervous system (ENS) is a part of the Peripheral nervous system (PNS) that controls the peristaltic activity of the gut wall which is essential for propulsion of food in the digestive tract. It is composed of a large number of neurons and glial cells, distributed throughout the length of the gut. These ganglion cells develop from the neural crest in the embryo. Failure of complete colonization of the gut by these enteric neural crest cells during early development of life results in absence of ganglia or neurons in a portion of the gut, usually the colon which leads to aperistaltis and severe intestinal obstruction. This is known as Hirschsprung’s disease (HSCR) also known as congenital megacolon. HSCR affects 1 in 4500 newborns \(^{(1, 2)}\). It appears either sporadically or has a familial basis and is often associated with other developmental defects. The main forms of treatment of HSCR are surgical resection of the aganglionic segment and pull through of the normal bowel. At present research is aimed at developing Cell based therapies for replacement of ganglion cells or enteric neuronal cells in the aganglionic portion of the gut thus aiming at restoring the function of the gut \(^{(1, 3, 5)}\). In this study we have isolated, in vitro expanded and characterized the Enteric Neuronal cells derived from human gut full thickness biopsy samples.

METHODS AND RESULTS

The postnatal gut full thickness biopsy samples of size 2-4 mm were obtained using from 13 patients undergoing gut resection surgery after informed consent. The samples were washed in Phosphate Buffer saline and using forceps, the outer smooth muscle layers along with the myenteric plexus were peeled off from the underlying tissue as strips. The strips were washed in Phosphate Buffer saline (PBS) and treated with 1mg/ml Collagenase/Dispase mixture in PBS for 30-45 min at 37°C. The digested cells were filtered with 70µm filter and the cell suspensions were centrifuged at 1800rpm for 10 mins. The pellet
obtained was suspended in DMEM/F12 medium supplemented with penicillin (100U/ml), streptomycin (100 µg/ml), L-glutamine (2 mmol/L), growth factors like bFGF (20ng/ml) and EGF (20ng/ml)\(^2\). Cell counting was done by Trypan Blue dye exclusion method and the cells were seeded in cell culture dishes coated with Fibronectin. The flasks with cells were incubated at 37°C with 5% CO2 for varying periods from 18 days-28 days. The cells were observed daily and media change was done every 2-3 days.

RESULTS

In all the samples, the Neurosphere like bodies (NLBs) were observed in the culture from 10th day onwards which were then subjected to histological and immunohistochemical studies. H&E staining showed positive for neural cells and Immunohistochemistry yielded positive for S-100, normally present in cells derived from the neural crest and Neuron Specific Enolase (NSE) a neuronal specific marker.

CONCLUSION:

We could successfully isolate and expand Human Enteric Neuronal cells from postnatal gut biopsy samples. Further research is warranted to utilize these Enteric Neuronal Cells for Cell based therapies to treat Hirschsprung’s disease.

REFERENCES


