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Review Article

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Recent Achievements in Stem Cell Therapy for Pediatric Gastrointestinal Tract Disease

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The field of stem cell research has been rapidly expanding. Although the clinical usefulness of research remains to be ascertained through human trials, the use of stem cells as a therapeutic option for currently disabling diseases holds fascinating potential. Many pediatric gastrointestinal tract diseases have defect in enterocytes, enteric nervous system cells, smooth muscles, and interstitial cells of Cajal. Various kinds of therapeutic trials using stem cells could be applied to these diseases. This review article focuses on the recent achievements in stem cell applications for pediatric gastrointestinal tract diseases. **(Pediatr Gastroenterol Hepatol Nutr 2013; 16: 10～16)**

Key Words: Stem cells, Tissue engineering, Gastrointestinal diseases, Child

INTRODUCTION

Stem cells are defined as the primitive and relatively unspecialized cells in fetal and adult tissues that have properties of self-renewal (longevity) and the ability to produce all the differentiated cell types of that tissue (multipotency) [1].

Stem cells can be classified as embryonic stem cells, bone marrow/hematopoietic stem cells, umbilical cord blood-derived very small embryonic-like stem cells [2], mesenchymal stem cells, inducible pluripotent stem cells, and tissue stem cells including intestinal enteric nervous system/epithelial stem cells [3]. On the basis of maturity, stem cells can be classified into adult (tissue) stem cells and immature stem cells including embryonic stem cells. Embryonic stem cells would be the best cells to use in clinical research, but their use raises ethical controversies.

To avoid graft rejection, which necessitates lifelong immunosuppression, and to avoid ethical issues, the potential for autologous transplantation is very important when stem cell transplantation is considered as a therapeutic option. Induced pluripotent stem cells and tissue stem cells such as enteric nerve system/epithelial stem cells meet this condition and these stem cells also have renewable sources.

There are several potential therapeutic applications of stem cells in the pediatric gastrointestinal

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diseases.

1) Restore tissue function: This is based on the ability of stem cells to differentiate into multiple cell types: enterocytes, hepatocytes, cardiomyocytes, osteocytes, chondrocytes, and adipocytes. Indications would include failure of the liver, small intestine, or pancreas [4-6].

2) Repair tissue: This is based on the paracrine function of stem cells that act as a source of secreted factors (vascular endothelial growth factor, insulin-like growth factor type 1, epidermal growth factor, etc.) that stimulate repair. Such stem cells could function as different stromal cells or directly modulate immune function [7].

3) Ameliorate disease activity: With mesenchymal stem cells, ameliorating disease is based on immunomodulation, which is mediated by prostaglandin E2, transforming growth factor-beta, and interleukin-10, and inhibition of inflammation, which is mediated by both innate and adaptive immune cells as well as active regulatory T cells by direct and indirect pathways (e.g., TSG-6). Indication would include inflammatory bowel disease or liver failure [8,9]. In addition, immunoablation and allogenic/autologous hemopoietic stem cell transplantation appear to be an effective treatment for some patients with Crohn's disease [10], including infants with early onset Crohn's disease [11].

Among these stem cell applications to pediatric gastrointestinal tract diseases, this review will focus on the restoration of tissue function for such diseases.

RESTORATION/REGENERATION OF GASTROINTESTINAL TRACT

The wall of gastrointestinal tract organs contains epithelial cells, circular and longitudinal smooth muscle layers, the enteric nervous system including the myenteric plexus, and the interstitial cells of Cajal (ICCs), which control the rhythmicity of contractions. Many pediatric gastrointestinal tract diseases involve damage to the enterocytes, enteric nervous system cells, smooth muscle (myocytes), and ICCs or all of these cell types. Short bowel syndrome (SBS) is the prototype of pediatric gastrointestinal disease, and of enterocyte disease, which can include gastric/intestinal ulcers, radiation enterocolitis, microvillous inclusion disease, and tufting enteropathy. Hirschsprung's disease is the prototype of enteric nervous system disease, which can include esophageal achalasia, internal anal sphincter (IAS) achalasia, hypertrophic pyloric stenosis, intestinal neuronal dysplasia, neuropathic intestinal pseudo-obstruction. Myopathic intestinal pseudo-obstruction is the prototype of smooth muscle disease. Abnormalities either in the number of ICCs or with the integrity of the ICC networks have been observed in various pediatric gastrointestinal diseases, including Hirschsprung's disease/total colonic aganglionosis, idiopathic gastric perforation, hypertrophic pyloric stenosis, transient neonatal pseudo-obstruction, neonatal meconium ileus, intestinal neuronal dysplasia, hypoganglionosis, internal anal sphincter achalasia, slow transit constipation (colonic inertia), diabetic gastroparesis, achalasia of the esophagus, chronic idiopathic intestinal pseudo-obstruction, paraneoplastic dysmotility, Chagas disease, and inflammatory bowel disease (ulcerative colitis, Crohn disease) [12].

Enteric nervous system (ENS) regeneration: Hirschsprung's disease

Normal neuromuscular function of the esophagus, stomach, small bowel, and colon requires the coordination of the tubular neuromuscular structures and the relevant sphincters. Hirschsprung's disease results from a congenital absence of ganglion cells in some part of the distal gut. The recent progress in using stem cells to restore defective ganglion cells offers new hope for the potential cure of Hirschsprung's disease.

Sources of ENS stem cells can be classified in various ways: Pluripotent stem cells can be harvested as embryonic stem cells, or induced pluripotent stem cells. Multipotent stem cells can be

harvested as central nervous system (CNS)-derived neural stem cells, non-CNS-derived neural stem cells or adult (tissue) ENS stem cells.

ENS neurosphere is defined as cellular aggregates containing both stem cells and their differentiated derivative cell types. Neurospheres were harvested from embryonic tissue [13], from post-natal human gut full-thickness tissue and post-natal human gut mucosal tissue (neonates, children, and adults) [14,15]. The most distinguished achievement in harvesting neurospheres is from autologous gut mucosal tissue by endoscopy. This allows for avoiding life-long immune suppression and provides a renewable source of ENS stem cells. Neurospheres were expanded with ex vivo culture and transplanted into a model of the aganglionic gut. Expansion and differentiation into neuron and glial cells was demonstrated [13-15]. Furthermore, neurons from transplanted neurospheres form synapses and regulate the contractility of the developing gut [16]. Intraperitoneally injected selected enteric neural crest stem cells engrafted diffusely throughout the postnatal gut of Hirschsprung's disease rats and differentiated into neurons and glia. Engraftment was not uniform, likely related to age-dependent changes in the gut mesenchyme [17]. Intraperitoneal injection is easily performed in sick neonates and may be developed as a technique for supplying exogenous ENS cells to the diseased postnatal gut. Smooth muscle protein from the aganglionic bowel increased neuronal survival and network formation of myenteric neurons and neural crest derived stem cells [18]. This implies that the microenvironment of stem cells is significant.

Interstitial cell of Cajal regeneration

ICCs are cells of mesodermal origin and generate unitary potential and slow waves. ICCs provide pacemaker activity to orchestrate gastrointestinal peristalsis and mixing. Tyrosine kinase receptor (c-Kit) and ANO 1 (TMEM16A), a membrane protein associated with calcium-dependent chloride channel are immunohistochemical markers of ICCs. The ultra-structural gold standard of ICCs includes close contacts established with nerve varicosities and the formation of numerous gap junctions, both with each other and with smooth muscle cells. The c-Kit signaling pathway, activated by stem cell factor (SCF), is the critical pathway associated with the control of ICCs survival and proliferation. In humans, the loss of ICC in motility disorders is nearly always associated with the loss of another cell type, including enteric nerve cells [12]. Neuronally derived NO (from neural nitric oxide synthase [nNOS]) modulates ICC numbers and network volume in the mouse gastric body. In NOS-/- mice, the remaining ICC cell bodies were often less well-formed and the processes blunted and decreased resulting in a disrupted network; this was most pronounced in the myenteric plexus region. Neuronally derived NO in particular is required for the maintenance of ICCs. NO appears to be a survival factor for ICCs [19]. Gastric relaxation in diabetics is hampered mainly by impaired NOS expression in the gastric myenteric plexus [20]. Long-standing diabetes mellitus may be associated with a decrease in a number of ICCs and a decrease in inhibitory innervations, associated with an increase in excitatory innervations. In murine diabetic gastroparesis, reduced SCF links smooth myopathy and loss of ICCs [21]. The restoration of ICC numbers and jejunal electrical rhythm, resulting from the blockade of the c-Kit signaling pathway, could be facilitated by local SCF administration in mice [22]. Exogenous SCF partially reversed the pathologic changes of ICC in the colon of diabetic mice [23].

Intestinal epithelium/mucosa repair

Up to now, three resources for epithelial/mucosal tissue regeneration have been identified, as follows: 1) from a single stem cell, stem cells can be regenerated with ex vivo expansion, 2) from a single intestinal crypt, intestinal crypts can be regenerated with ex vivo expansion, 3) from tissue engineering, intestinal tissue can be regenerated with a scaffold and stem cells.

1. Single stem cell use

Since Lgr5 was identified as a definitive marker of crypt stem cells in the small intestine and colon [24], single stem cells build crypt-villus structures in vitro without a mesenchymal niche [25]. The mouse gastric unit [26], and human epithelial organoids of the colon [27,28] were built in vitro and kept for a long period of time. Recently, dextran sulfate sodium-induced local colonic injury was treated with a stem cell rectal enema and functional engraftment of the colonic epithelium was observed. These stem cells were expanded in vitro from a single adult Lgr5+ stem cells [29].

2. Single intestinal crypt use

Intestinal crypts reproducibly expand in culture [30]. This study showed that the proximal jejunum tends to form enteroids more efficiently than the distal ileum. When enteroid forming efficiency following freeze-thaw was tested, enteroid morphology and crypt budding was maintained as prior to freezing. This study holds tremendous promise for future therapeutic usage.

Organ repair of conditions such as SBS

The above-mentioned two methods usually focus on epithelial regeneration. However, SBS is a tragic status affecting all of the components of the intestine. For SBS, more than epithelial regeneration is needed. In tissue engineering, stem cells or organoid units (multicellular clusters with predominantly epithelial contents in case of SBS) were loaded onto a scaffold, and part of the organ could be regenerated. In brief, animal organs are decellularized to obtain acellular extracellular matrix (ECM) scaffolds (all molecular components [collagens, elastin, fibronectin, laminin, glycosaminoglycans, etc.] preserved, as well as essential growth factors that are present within the ECM scaffold). Cells are harvested from the patient, expanded, and seeded onto or into the scaffold. This construct is allowed to mature in a bioreactor. After maturation, it is implanted in the patient [31].

Outcomes of transplantation of the whole liver or pancreas are far better than those of transplantation of liver cells or pancreatic islets alone [31]. The significance of the stem cell niche (signals provided by these cellualr and acellular components appear to be integrated by stem cells to inform their fate decision, self renewal or differentiation, migration or retention, and cell death or survival) should be evaluated from this perspective.

Autologous tissue-engineered small intestine from ex vivo expansion enables avoiding the problems of transplants, immunosuppression, and donor supply. In a rat study, a tissue- engineered small intestine was formed by transplanting donor organoid units on a polymer scaffold into a host [32]. SBS-induced rats regained a higher percentage of preoperative weight with intestinal organoids using tissue-engineered small intestine than those without tissue engineered small intestine [33]. The tissue-engineered small intestine from autologous tissue in a large animal model showed successful differentiation into goblet cells, enteroendocrine cell, smooth muscle and ganglion cells in Auerbach's and Meisner's plexus and stem cells [34].

Animal experimental trials for esophageal replacement with tissue engineering have been performed. A tissue engineered esophageal construct may be created by the combination of a scaffold and stem cells [35]. In a canine study, an implanted tissue-engineered esophagus showed distensibility, but no peristaltic contractions. Shrinkage of the keratinocytes and smooth muscle also resulted in a shorter segment of restored esophagus [36]. This can be applied to esophageal atresia with insufficient length for primary anastomosis.

A tissue-engineered stomach can replace a native stomach in a rat model. Replacement of the native stomach by a tissue-engineered stomach had beneficial effects on the formation of neomucosa and smooth muscle layers in the tissue-engineered stomach [37]. A tissue-engineered stomach has the potential to function as a food reservoir following

total gastrectomy in a rat model [38].

Transplantation of neural stem cells to the pylorus showed improved relaxation of pylorus muscle strips and improved gastric emptying in nNOS deficient mice [39].

A tissue-engineered rat large intestine can be successfully produced with fidelity to the native architecture and in vitro function from neonatal syngeneic tissue, adult tissue, and tissue-engineered colon itself [40].

The ring-shaped construction of the IAS was bioengineered in vitro from isolated smooth muscle cells in rabbits [41], in humans [42], and in mice [43], and bioengineered IAS rings demonstrate physiological functionality [41-43]. Bioengineered IAS rings were implanted subcutaneously and successfully grew and survived with respect to viability and functionality in mice [44]. Human IAS circular smooth muscle was co-cultured with immortomouse fetal enteric neurons. Implanted, intrinsically innervated bioengineered human IAS tissue developed neovascularization, myogenic tone, and normal contraction and relaxation characteristics in response to testing in mice [45]. These studies might pave the way for combining the enteric nervous and gastrointestinal smooth muscle components that will be critical in providing treatment for clinical neuromuscular diseases such as fecal incontinence.

CONCLUSION

The gastrointestinal tract is a complex series of specialized neuromuscular tubes. For SBS, in addition to the mucosal component, additional challenges due to the vascular component and the peristaltic function of the muscular and neural components should be held. More studies are needed to understand the biology of stem cells, and assess stem cell oncogenic properties. Consensus on the best methods to use for stem cell purification, the ideal route of delivery, the amount of cells to infuse, and the timing of infusions, are required.

Many challenges for regenerative medicine approaches remain: identifying sources for cells, construction of scaffolds that result in the proper three-dimensional growth of the selected cells into the desired organ, physiological orientation of the component layers of the wall of the gastrointestinal organs, and the functional integration of the key cells: smooth muscle, enteric nerves, and ICCs [46]. We also feel that without genetic modification or the ability to manipulate the immune system, autologous cells and/or stem cell-derived organs will not necessarily correct diseases resulting from genetic disorders, or diseases caused by an impairment of the immune system. Mesenchymal stem cells deserve attention from this perspective.

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