Published in final edited form as:

J Pediatr Surg. 2019 March ; 54(3): 413–416. doi:10.1016/j.jpedsurg.2018.07.025.

Potential Role of Stem Cells in Disease Prevention Based on a Murine Model of Experimental Necrotizing Enterocolitis

Courtney Pisano, DO, MS and Gail E. Besner, MD

Department of Pediatric Surgery, Center for Perinatal Research, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH, 43205, USA

Abstract

BACKGROUND: Necrotizing enterocolitis (NEC) is a devastating disease of newborns, and despite years of research, there is no known cure. The mortality rate of infants with NEC remains as high as 20–30%. Babies who survive NEC frequently have long term complications including short gut syndrome, developmental delays and neurological sequelae. Unfortunately, despite much research over the past years, the precise pathogenesis of the disease is still not completely understood.

METHODS: Our laboratory has focused on identifying novel therapies to prevent the disease, including the use of stem cells (SC), heparin-binding epidermal growth factor-like growth factor (HB-EGF) and recently, stem cell derived-exosomes, a type of nanovesicle, to combat this illness.

RESULTS: We have outlined the major SC lines and data suggesting potential benefit as a curative or preventive approach for NEC as well as describing several new therapeutic strategies, including stem cell derived-exosomes and HB-EGF for decreasing the incidence and severity of this disease in rat models in our lab.

CONCLUSION: Overall, our lab has demonstrated that these different types of SC equivalently reduce the incidence and severity of NEC and equally preserve intestinal barrier function during NEC. We have previously demonstrated that AF-MSC can protect the intestines from intestinal injury and may therefore hold strong therapeutic potential for the prevention of NEC. Most recently, our work with stem cell derived-exosomes have shown to be equivalent to their derived SC lines in decreasing the incidence of this disease.

Keywords

Necrotizing enterocolitis; stem cells; intestine; injury; exosomes

Corresponding author: Gail E. Besner, MD, Department of Pediatric Surgery, FB6135, Nationwide Children's Hospital, 700 Children's Drive, Columbus, Ohio 43205, USA, gail.besner@nationwidechildrens.org, Phone: (614) 722-3930, FAX: (614) 722-3903.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Necrotizing enterocolitis (NEC) continues to be a primary cause of morbidity and mortality among infants, especially those born prematurely (1). Although the exact pathogenesis of this disease is unknown, there are several recognized characteristics of infants who develop NEC, including differences in the intestinal microbiome (3,4,5) as well as a heightened inflammatory response (6). Among several experimental strategies designed to cure or prevent the disease, stem cell (SC) therapy remains an attractive possibility. Stem cells have the ability to differentiate, self-replicate, prevent apoptosis and reduce inflammation. Additionally, they have been shown in numerous disease models to lead to improvements in tissue health and function (7–9). Due to promising findings and advances in SC research, increased attention has focused on SC as a potential future therapy for NEC.

Types of Stem Cells

Our laboratory has investigated the ability of different types of SC and their secreted products to protect the intestines from NEC. All of these cells have distinctive characteristics that allow them to be specifically identified and their identities confirmed (Table 1). The stem cell lines used in our lab are described below in further detail.

Bone marrow-derived mesenchymal stem cells (BM-MS C)

Bone marrow-derived mesenchymal stem cells are readily derived from harvesting marrow from the long bones of donor subjects and placing it into culture to select for MSC (11). These cells must be passaged several times to minimize contamination with hematopoietic precursor cells which are present in the original sample. To confirm that these are indeed MSC, the cells are examined for the presence of CD44 and CD90 as well as the absence of cells expressing CD45 (11). We have examined both intraperitoneal (IP) and intravenous (IV) routes of administration of BM-MS C in rat and mouse NEC models. BM-MS C derived from mice, rats and humans have been shown to be equally effective in reducing the incidence and severity of NEC in these models (11–14). However, many cells become entrapped in the lungs after IV injection, leading to a reduction in the number of cells available for therapeutic purposes (14–16). This finding may be important when determining the best route of administration of these stem cells in the clinical setting.

Amniotic fluid-derived mesenchymal stem cells (AF-MS C)

Amniotic fluid-derived mesenchymal stem cells can be collected and cultured from AF samples during routine amniocentesis or caesarean section (18,19). AF-MS C have very promising properties, including ease of collection and culture. They can be cultured *in vitro* with few media supplements and grow very rapidly. They grow significantly faster and can be more easily and readily cultured than BM-MS C (11). There has been much interest in these cells, as they have been shown to significantly reduce the incidence and severity of NEC in a murine model after IP injection (20). Additional studies using IP injection of AF-MS C have demonstrated that these cells significantly decrease histologic injury and lead to improved gut barrier function during experimental NEC (11,21).

Amniotic Fluid-derived Neural Stem Cells (AF-NSC)

Amniotic fluid-derived neural stem cells can be cultured and isolated from AF and selectively grown. These cells do not grow as quickly as MSC and have more complicated culture requirements (11). AF-NSC are placed into slightly different culture media than MSC—the presence of epidermal growth factor (EGF) and fibroblast growth factor (FGF) helps to ensure that these NSC remain in an undifferentiated state (11). As these SC grow and develop, they aggregate into neurospheres. These neurospheres can be mechanically separated to obtain individual NSC. However, these cells do not grow as quickly as MSC and it may take weeks to collect substantial numbers of cells. The expression of nestin confirms the identity of these cells. Nestin is an intermediate filament protein present in developing NSC but downregulated when the cells become mature neural cells (25). AF-NSC have been shown to decrease the severity and incidence of NEC in experimental models when injected IP (11).

Enteric Neural Stem Cells (E-NSC)

The enteric nervous system (ENS) contains a very high concentration of nerve endings and NSC, located within the gut (26). Enteric neural stem cells are derived from the gut and have similar properties to AF-NSC. The culture medium for these cells is the same as that used to grow NSC. E-NSC are challenging to obtain, and the cells must be put through a combination of mechanical and enzymatic digestion of the gut to obtain the cells from the muscular layer of the small intestine (11). After digestion, these cells are placed into culture to allow NSC to replicate and form neurospheres. These neurospheres are then mechanically separated into individual NSC, confirmed by nestin expression (11,25). Our lab, as well as several others, have shown that E-NSC are beneficial in treating experimental NEC and improving gut barrier function in animal models (11, 21, 24, 27).

Enteric Nervous System Damage During NEC and the Role of Neurotransplantation in Established NEC

The ENS is a highly organized part of the autonomic nervous system that innervates the entire gastrointestinal tract by several interconnected neuronal networks. The ENS has the capacity to adapt to microenvironmental influences and it continues to change significantly throughout its lifespan (44). The ENS is organized into two major ganglionated networks, the myenteric and the submucous plexuses and into several aganglionated plexuses within the mucosa and muscularis, and underneath the serosa (45).

During the development of NEC, there are significant changes to the ENS. We have previously identified ENS abnormalities in NEC-afflicted human intestine (24). We further used an animal model of NEC to confirm that these abnormalities persist long after the acute onset of NEC has resolved (24). When comparing human NEC intestine with control intestine, there was significant ENS damage with increased cellular apoptosis and decreased neuronal nitric oxide synthase expression in myenteric ganglia. Similar abnormalities were identified in rat pups exposed to experimental NEC (24).

Like the central nervous system, neurons in the ENS have restricted potential for regeneration after injury. This may lead to a failure to reverse the neuronal cell loss that occurs during NEC, which in turn could lead to compromised intestinal function long after recovery from the acute disease process. Based on these findings, we examined transplantation of intestinal enteric neurons to restore the neuronal cell loss associated with NEC (24). When E-NSC were administered to rat pups with established experimental NEC, engraftment of the transplanted NSC into injured intestine was demonstrated and there were improvements in ENS integrity, intestinal motility and survival. These experiments suggest the potential of E-NSCs as a future treatment for established NEC (24).

Mechanisms of Stem Cell Activity

Regarding the mechanisms of intestinal protection from NEC, SCs generate an array of cytokines, growth factors, miRNAs, and extracellular vesicles that may be responsible for their effects on mitigating NEC-related injury in these experimental models. Studies addressing this are ongoing. Others have demonstrated that interleukin 6 (IL-6), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are released by MSCs via paracrine mechanisms, which leads to enhanced intestinal epithelial viability and proliferative capacity after hypoxic injury (48,49,50). There have also been several experimental models in which rodents and pigs were treated with whole amniotic fluid (AF) without purification of stem cells. These studies demonstrated significant protection from experimental NEC. Interestingly, concentrations of stem cells in AF are usually low, but AF appears to have significant protective effects on the intestines in these models. This information may be useful going forward, as a small amount of AF may have a great impact on this disease (22,47,50). This information may be important in translating this research to the bedside. Based on our results, harvesting and preservation of amniotic fluid at the time of amniocentesis or delivery would be reasonable in the future if costs were not prohibitive.

Summary of Stem Cell Findings

Overall, our lab has demonstrated that these different types of SC equivalently reduce the incidence and severity of NEC (11) and equivalently preserve intestinal barrier function during NEC (Figure 1) (21). We have previously demonstrated that AF-MSC can protect the intestines from intestinal injury and may therefore hold strong therapeutic potential for the prevention of NEC (12, 34). Clinically, amniotic fluid is easily obtainable at the time of delivery and AF-MSC could be harvested and maintained. In addition, we have demonstrated the potential of E-NSCs as a rescue therapy for established NEC. Given that current therapies for NEC (gut rest, broad spectrum antibiotics and resection of dead bowel) have not changed significantly in four decades, this represents a promising breakthrough. While the exact mechanisms remain unclear, we are currently studying engraftment rates between different stem cell populations as well as examining SC-derived exosomes as a means of non-cell based treatments for NEC.

Initially, we thought that engraftment of administered stem cells into the intestine, either before or after injury, would be necessary to achieve efficacy. However, while engraftment rates have consistently been low, SC efficacy remained high (21, 35). This finding led us to

explore the hypothesis that the major effect of SC administration in NEC prevention is not engraftment but paracrine or endocrine secretion of factors, exosomes or vesicles.

Exosomes

Exosomes are extracellular nanovesicles ~ 40–100 nm in size containing DNA, RNA, microRNA, and proteins, that are released by all cells via exocytosis. They are secreted by different cell types into the extracellular space and then fuse with the cell membranes of other host cells (12, 36). In this way, exosomes act as intracellular messengers that can affect changes in both neighboring and remote cells throughout the body (30). One of the mechanistic characteristics of exosomes is a negative charge shown by analysis of ζ -potential, which allows their interaction with positively charged molecules (46). Exosomes have emerged over the last several years as a very important mechanism of cell communication, and are implicated in wound healing and cellular growth (12,31–33). Exosomes secreted by MSC have been shown to contain miRNAs that are anti-apoptotic and promote wound healing and angiogenesis (12, 36). Previously, we have shown that exosomes derived from BM-MSC protect the intestines and preserve gut barrier function in experimental NEC as effectively as the SC from which they are derived. In this study, we compared the effects of BM-MSC-derived exosomes to the effects of administering BM-MSC themselves, and found both to be equally effective at decreasing NEC incidence, NEC severity, and gut permeability. Most importantly, these results suggest that exosomes are the main paracrine effectors of these cells, and may be principally responsible for the ability of BM-MSC to protect the intestines from NEC (12).

These findings led us to further investigate the ability of exosomes from other types of stem cells to protect the intestines from NEC. Exosomes were derived from BM-MSC, AF-MSC, AF-NSC and enteric NSC. We found that exosomes from all of these SC types demonstrated a significant reduction in NEC (Figure 2) (35). Thus, treatment with SC-derived exosomes is equivalent to treatment with the SC from which these exosomes are derived (35). Dose-response studies demonstrated that exosomes administered at concentrations of 8.0×10^7 or 4.0×10^8 exosomes/50 μ l had the greatest effects (35)

Since exosomes do not trigger the same immunogenic response as SC due to their size and mechanism of action, they present two additional advantages over SC (36,37). First, exosomes do not carry the same theoretical potential concern for tumorigenicity as do SC themselves, which may have particular value when treating infants (who we anticipate will have a long lifespan). Additionally, due to their small size, SC-derived exosomes have been shown to be beneficial in several animal models of traumatic brain injury, since they can cross the blood-brain barrier, unlike the SC from which they are derived (38). These findings are very encouraging for NEC research, as patients who develop NEC may have subsequent neurological dysfunction due to their disease. While the exact mechanisms by which exosomes exert effects across the blood brain barrier are not fully understood, the hypothesis that SC-derived exosomes could treat or prevent both NEC and the associated brain injuries of premature infants is intriguing (35, 38,39).

Augmenting SC-Derived Exosomes

While we have shown that stem cell-derived exosomes significantly decrease the incidence and severity of NEC (35), it is desirable to further optimize exosome treatment. One promising approach is to augment exosome cargo with additional beneficial components such as heparin-binding epidermal growth factor-like growth factor (HB-EGF) to improve exosome-mediated therapy. HB-EGF is a member of the epidermal growth factor (EGF) family (40). It is a potent mitogen and chemotactic factor, and we have previously demonstrated that enteral administration of HB-EGF protects the intestines from injury (14, 41). HB-EGF promotes enterocyte migration and proliferation and preserves intestinal stem cell viability during experimental NEC (42,43). We have demonstrated in our NEC rat model that NSC alone or HB-EGF alone significantly decreased intestinal permeability, with a further decrease in intestinal permeability with simultaneous administration of HB-EGF and NSC. We also demonstrated a significant decrease in intestinal permeability with administration of HB-EGF-overexpressing NSC compared with non-transfected NSC. (14). Based on these findings, augmenting the efficacy of exosomes with the additional cargo of HB-EGF may further boost protection against NEC. We anticipate testing this hypothesis in both our standard NEC-prevention rodent model and our novel treatment-of-established-NEC model.

Clinical SC Trials

MSCs are being examined for efficacy in various disease processes. Over 500 clinical trials investigating this have been registered in the www.ClinicalTrials.gov database (51). While there have been no clinical trials on the use of SC to prevent NEC to date, it is feasible that clinical trials may take place in the future based on the success of experimental models using small and large animals. The idea of using exosomes in clinical trials related to vaccinations started almost two decades ago. While investigating the role of these exosomes, researchers found that exosomes derived from dendritic cells induced rejection of tumors in rodent models. This rejection involved activation of tumor-specific cytotoxic T cells. This discovery lead to several clinical trials, including a Phase I anti-non-small cell lung cancer clinical trial in the United States (50). Exosomes and extracellular vesicles are being used in a number of clinical trials for various diseases throughout the world. Based on success from these trials, and the positive results we have obtained in our laboratory, we believe that clinical trials involving SC-derived exosomes in NEC is feasible in the future.

Acknowledgements:

This work was supported by NIH R01 GM113236

References

1. Neu J, Walker WA. Necrotizing Enterocolitis. N Engl J Med 2011;364(3):255–264. doi:10.1056/NEJMra1005408. [PubMed: 21247316]
2. Herrmann K, Carroll K. An Exclusively Human Milk Diet Reduces Necrotizing Enterocolitis. Breastfeed Med 2014;9(4):184–190. doi:10.1089/bfm.2013.0121. [PubMed: 24588561]

3. Li Q-Y, An Y, Liu L, et al. Differences in the Clinical Characteristics of Early- and Late-Onset Necrotizing Enterocolitis in Full-Term Infants: A Retrospective Case-Control Study. *Sci Rep* 2017;7:43042. doi:10.1038/srep43042. [PubMed: 28211488]
4. Coggins SA, Wynn JL, Weitkamp J-H. Infectious Causes of Necrotizing Enterocolitis. *Clin Perinatol* 2015;42(1):133–154. doi:10.1016/j.clp.2014.10.012. [PubMed: 25678001]
5. Warner BB, Deych E, Zhou Y, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. *Lancet* 2016;387(10031):1928–1936. doi: 10.1016/S0140-6736(16)00081-7. [PubMed: 26969089]
6. Sharma R, Tepas JJ, Hudak ML, et al. Neonatal gut barrier and multiple organ failure: role of endotoxin and proinflammatory cytokines in sepsis and necrotizing enterocolitis. *J Pediatr Surg* 2007;42(3):454–461. doi:10.1016/j.jpedsurg.2006.10.038. [PubMed: 17336180]
7. Galindo LT, Filippo TRM, Semedo P, et al. Mesenchymal stem cell therapy modulates the inflammatory response in experimental traumatic brain injury. *Neurol Res Int* 2011;2011:564089. doi:10.1155/2011/564089. [PubMed: 21766025]
8. Rowart P, Erpicum P, Detry O, et al. Mesenchymal Stromal Cell Therapy in Ischemia/Reperfusion Injury. *J Immunol Res* 2015;2015:1–8. doi:10.1155/2015/602597.
9. Diaco N, Diamandis Z, Borlongan C. Amniotic fluid-derived stem cells as an effective cell source for transplantation therapy in stroke. *Brain Circ* 2015;1(2):119. doi:10.4103/2394-8108.172881.
10. Bongso A, Richards M. History and perspective of stem cell research. *Best Pract Res Clin Obstet Gynaecol* 2004;18(6):827–842. doi:10.1016/j.bpobgyn.2004.09.002. [PubMed: 15582541]
11. McCulloh CJ, Olson JK, Zhou Y, Wang Y, Besner GE. Stem Cells and Necrotizing Enterocolitis: A Direct Comparison of the Efficacy of Multiple Types of Stem Cells. *J Pediatr Surg* 2017;In Press.
12. Rager TM, Olson JK, Zhou Y, Wang Y, Besner GE. Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotizing enterocolitis. *J Pediatr Surg* 2016;51(6):942–947. doi:10.1016/j.jpedsurg.2016.02.061. [PubMed: 27015901]
13. Tayman C, Uckan D, Kilic E, et al. Mesenchymal Stem Cell Therapy in Necrotizing Enterocolitis: A Rat Study. *Pediatr Res* 2011;70(5):489–494. doi:10.1203/PDR.0b013e31822d7ef2. [PubMed: 21772224]
14. Yang J, Watkins D, Chen C-L, Bhushan B, Zhou Y, Besner GE. Heparin-Binding Epidermal Growth Factor-Like Growth Factor and Mesenchymal Stem Cells Act Synergistically to Prevent Experimental Necrotizing Enterocolitis. *J Am Coll Surg* 2012;215(4):534–545. doi:10.1016/j.jamcollsurg.2012.05.037. [PubMed: 22819639]
15. Furlani D, Ugurlucan M, Ong L, et al. Is the intravascular administration of mesenchymal stem cells safe? *Microvasc Res* 2009;77(3):370–376. doi:10.1016/j.mvr.2009.02.001. [PubMed: 19249320]
16. Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem Cell Transplantation: The Lung Barrier. *Transplant Proc* 2007;39(2):573–576. doi:10.1016/j.transproceed.2006.12.019. [PubMed: 17362785]
17. Anker PS. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood* 2003;102(4):1548–1549. doi:10.1182/blood-2003-04-1291. [PubMed: 12900350]
18. Bottai D, Cigognini D, Nicora E, et al. Third trimester amniotic fluid cells with the capacity to develop neural phenotypes and with heterogeneity among sub-populations. *Restor Neurol Neurosci* 2012;30(1):55–68. doi:10.3233/RNN-2011-0620. [PubMed: 22377907]
19. Murphy S, Atala A. Amniotic Fluid and Placental Membranes: Unexpected Sources of Highly Multipotent Cells. *Semin Reprod Med* 2013;31(1):062–068. doi:10.1055/s-0032-1331799.
20. Zani A, Cananzi M, Fascetti-Leon F, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. *Gut* 2014;63(2):300–309. doi:10.1136/gutjnl-2012-303735. [PubMed: 23525603]
21. McCulloh CJ, Olson JK, Wang Y, Vu J, Gartner S, Besner GE. Evaluating the Efficacy of Different Types of Stem Cells in Preserving Gut Barrier Function in NEC. *J Surg Res* 214:278–285, 6 2017 Epub March 31,2017. [PubMed: 28624056]

22. Siggers J, Ostergaard MV, Siggers RH, et al. Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates. *AJP Gastrointest Liver Physiol* 2013;304(10):G864–G875. doi:10.1152/ajpgi.00278.2012.
23. Good M, Siggers RH, Sodhi CP, et al. Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. *Proc Natl Acad Sci* 2012;109(28):11330–11335. doi: 10.1073/pnas.1200856109. [PubMed: 22733781]
24. Zhou Y, Yang J, Watkins DJ, et al. Enteric nervous system abnormalities are present in human necrotizing enterocolitis: potential neurotransplantation therapy. *Stem Cell Res Ther* 2013;4(6): 157. doi:10.1186/scrt387. [PubMed: 24423414]
25. Lendahl U, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. *Cell* 1990;60(4):585–595. [PubMed: 1689217]
26. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* 2011;12(8):453–466. doi:10.1038/nrn3071. [PubMed: 21750565]
27. Wei J, Zhou Y, Besner GE. Heparin-binding EGF-like growth factor and enteric neural stem cell transplantation in the prevention of experimental necrotizing enterocolitis in mice. *Pediatr Res* 2015;78(1):29–37. doi:10.1038/pr.2015.63. [PubMed: 25806717]
28. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc cell Biol* 2006;Chapter 3:Unit 322. doi: 10.1002/0471143030.cb0322s30. [PubMed: 18228490]
29. Lo Sicco C, Reverberi D, Balbi C, et al. Mesenchymal Stem Cell-Derived Extracellular Vesicles as Mediators of Anti-Inflammatory Effects: Endorsement of Macrophage Polarization. *Stem Cells Transl Med* 2017;6(3):1018–1028. doi:10.1002/sctm.16-0363. [PubMed: 28186708]
30. Edgar JR, Harding C, Heuser J, et al. Q&A: what are exosomes, exactly? *BMC Biol*, 14 (2016), p. 46 [PubMed: 27296830]
31. Harding CV, Heuser JE, Stahl PD. Exosomes: looking back three decades and into the future. *J Cell Biol* 2013; 200:367–71. [PubMed: 23420870]
32. De Jong OG, Van Balkom BW, Schiffelers RM, et al. Extracellular vesicles: potential roles in regenerative medicine. *Front Immunol* 2014; 5:608. [PubMed: 25520717]
33. Qin J, Xu Q. Functions and application of exosomes. *Acta Pol Pharm* 2014; 71:537–43. [PubMed: 25272880]
34. Watkins DJ, Zhou Y, Matthews MA, et al. HB-EGF augments the ability of mesenchymal stem cells to attenuate intestinal injury. *J Pediatr Surg* 2014;49:938–44 [discussion 944]. [PubMed: 24888839]
35. McCulloh Olson, et al. Treatment of necrotizing enterocolitis with Stem cell-derived exosomes; In Press
36. van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a specialized function. *J Biochem* 2006 7; 140(1):13–21 [PubMed: 16877764]
37. Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles* 2014;3:24641. doi:10.3402/jev.v3.24641.
38. Xiong Y, Mahmood A, Chopp M. Emerging potential of exosomes for treatment of traumatic brain injury. *Neural Regen Res* 2017;12:19. doi:10.4103/1673-5374.198966 [PubMed: 28250732]
39. Derkus B, Emregul KC, Emregul E. A new approach in stem cell research-Exosomes: Their mechanism of action via cellular pathways. *Cell Biol Int* 2017;41:466–75. doi:10.1002/cbin.10742. [PubMed: 28191696]
40. Higashiyama S, Abraham JA, Miller J, Fiddes JC, Klagsbrun M. A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science* 1991; 251:936–9. [PubMed: 1840698]
41. Feng J, El-Assal ON, Besner GE. Heparin-binding epidermal growth factor-like growth factor decreases the incidence of necrotizing enterocolitis in neonatal rats. *J Pediatr Surg* 2006;41:144–9; discussion 9.
42. Feng J, Besner GE. Heparin-binding epidermal growth factor-like growth factor promotes enterocyte migration and proliferation in neonatal rats with necrotizing enterocolitis. *J Pediatr Surg* 2007;42:214–20. [PubMed: 17208569]

43. Chen CL, Yu X, James IO, et al. Heparin-binding EGF-like growth factor protects intestinal stem cells from injury in a rat model of necrotizing enterocolitis. *Lab Invest* 2012;92:331–44. [PubMed: 22157721]
44. Scahfer K, Van Ginneken C, Copray S. Plasticity and Neural Stem Cells in the Enteric Nervous System. *The Anatomical Record* 292:1940–1952. 2009. [PubMed: 19943347]
45. Timmermans JP, Adriaenssens D, Scheuermann D. Structural Organization and Neuropeptide Distribution in the Mammalian Enteric Nervous System, with Special Attention to Those Components Involved in Mucosal Reflexes. *Comparative Biochemistry and Physiology Part A: Physiology* Volume 118, Issue 2: 331–340. 1997.
46. Deregibus MC, Figliolini F, et al. Charge-based precipitation of extracellular vesicles. *Int J Mol Med* 2016 11; 38(5):1359–1366. [PubMed: 28025988]
47. Zani Augusto, Cananzi M, Fascetti-Leon F, et al. “Amniotic Fluid Stem Cells Improve Survival and Enhance Repair of Damaged Intestine in Necrotizing Enterocolitis via a COX-2 Dependent Mechanism.” *Gut*, vol 63, no. 2, 2013, pp. 300–309., doi:10.1136/gutjnl-2012-303735.
48. Bruno S, Collino F, Tetta C, Camussi G. Dissecting the paracrine effectors for mesenchymal stem cells. *Adv Biochem Eng Biotechnol* 2013;129:137–152. [PubMed: 22968371]
49. Weil BR, Markel TA, Herrmann JL, Abarbanell AM, Meldrum DR. Mesenchymal stem cells enhance the viability and proliferation of human fetal intestinal epithelial cells following hypoxic injury via paracrine mechanisms. *Surgery* 2009;146(2): 190–197. [PubMed: 19628073]
50. Drucker Natalie A., McCulloh CJ., Li Bo, Pierro A., Besner GE, Markle T “Stem Cell Therapy in Necrotizing Enterocolitis: Current State and Future Directions.” *Seminars in Pediatric Surgery*, vol. 27, no. 1, 2018, pp. 57–64., doi:10.1053/j.sempedsurg.2017.11.011. [PubMed: 29275819]
51. Lener Thomas, et al. “Applying Extracellular Vesicles Based Therapeutics in Clinical Trials â an ISEV Position Paper.” *Journal of Extracellular Vesicles*, vol. 4, no. 1, 2015, p. 30087., doi: 10.3402/jev.v4.30087. [PubMed: 26725829]

Fig. 1.

Administration of SCs decrease the incidence and severity of experimental NEC.

Percentages of pups with incidence of grades 2, 3, 4 NEC after IP injection of either PBS or 2 million SCs in 100 μ l. Histologic grade is based on previously described criteria [18]. Bold numbers at the top of each bar represent total incidence of NEC in that treatment group. White numbers in colored subsections indicate percentage of corresponding NEC grade.

Fig. 2.

Administration of SC-derived exosomes decrease the incidence and severity of experimental NEC. Percentages of pups with incidence of grades 2, 3, 4 NEC incidence after IP injection of 8×10^7 exosomes [29]. Bold numbers at the top of each bar represent total incidence of NEC in that treatment group. White numbers in colored subsections indicate percentage of corresponding NEC grade.

Table 1.**Stem Cell Types and Cellular Characteristics**

Type of Stem Cell (SC)	Source	Cellular Characteristics
Bone marrow-derived mesenchymal stem cells (BM-MSC)	Marrow from long bones	<ul style="list-style-type: none"> • must be cultured and passaged several times to minimize contamination with hematopoietic precursors • verification as BM-MSC includes positive expression of CD44 and negative expression of CD45 (11–14)
Amniotic fluid-derived mesenchymal stem cells (AF-MSC)	Amniotic fluid	<ul style="list-style-type: none"> • expression of surface markers such as CD29, CD44, and CD90 • include stage-specific embryonic antigen (SSEA)-4 and the transcription factor Oct4, which help to ensure that the cells remain undifferentiated (11,18,19)
Amniotic fluid-derived neural stem cells (AF-NSC)	Amniotic fluid	<ul style="list-style-type: none"> • form neurospheres containing predominantly NSC in culture (25) • express nestin, an intermediate filament present in developing NSC but downregulated when the cells become mature neural cells (25)
Enteric neural stem cells (E-NSC)	Muscular layer of the small intestine	<ul style="list-style-type: none"> • form neurospheres predominantly in culture (25) • express markers specific for NSC including nestin once mechanically separated to yield individual NSC (11,25)