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Treatment of experimental necrotizing enterocolitis with stem cell-derived exosomes

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ABSTRACT

Purpose: Necrotizing enterocolitis (NEC) remains a devastating disease in premature infants. We previously showed that four stem cell (SC) types equivalently improve experimental NEC. Exosomes are intercellular nanovesicles containing RNA, miRNA, DNA, and protein. Because SC therapy faces challenges, our aim was to determine if the beneficial effects of SC are achievable with cell-free exosomes.
Methods: Exosomes from four SC types were compared: (1) amniotic fluid-derived mesenchymal SC (AF-MSC); (2) bone marrow-derived MSC (BM-MSC); (3) amniotic fluid-derived neural SC (AF-NSC); and (4) neonatal enteric NSC (E-NSC). Rat pups exposed to NEC received a varying concentration of a single type of exosome with control pups receiving PBS only. Intestinal damage was graded histologically.
Results: The incidence of NEC was 0% in unstressed pups and 60.7% in control pups subjected to NEC. Exosomes (4.0×10^8) reduced NEC incidence to: AF-MSC 25.0%; BM-MSC 23.1%; AF-NSC 11.1%; E-NSC 27.3%. When administered at a concentration of at least 4.0×10^8, all groups demonstrated a significant reduction in NEC compared to untreated pups. At this minimum concentration, there was no difference in treatment efficacy between exosomes and the SC from which they were derived.
Conclusion: Stem cell-derived exosomes reduce the incidence and severity of experimental NEC as effectively as the stem cells from which they are derived, supporting the potential for novel cell-free exosome therapy for NEC.
Type of study: Basic science.

Necrotizing Enterocolitis (NEC) has been the subject of a substantial amount of research; however, little progress has been made in significantly improving patient outcomes, with overall mortality for infants requiring surgery remaining greater than 30% [1]. The cost of treating premature infants affected with NEC exceeds $1 billion annually in the United States alone. Morbidity and mortality remain unacceptably high and have been relatively unchanged in several decades [2]. We have previously shown that four types of stem cells all equivalently promote improved gut barrier function and reduce the incidence and severity of experimental NEC [3,4].

Translation of stem cell research to the clinic is challenging, with many ethical, legal, and scientific ramifications [5]. Interest has emerged in the potential diagnostic and therapeutic use of exosomes, small extracellular vesicles approximately 100 nm in size [6]. Exosomes are exocytosed by cells and contain RNA, miRNA, DNA, and proteins that are not only reflective of intracellular activity in the cells from which they were derived, but also are able to affect changes in neighboring cells and remote cells throughout the body via hematogenous spread [7]. These nanovesicles have been shown to have therapeutic potential in a number of areas, and interest has become so strong that in 2012 the NIH established a Common Fund initiative specifically to fund research into exosome origins, distribution, and potential impact [8–10]. In the current study, we sought to determine if stem cell-derived exosomes have potential as a cell-free therapy in NEC, and if so, to determine if the therapeutic effects are dose-dependent.

1. Methods

1.1. Stem cell culture and verification

Using previously described methods, the following stem cell lines were cultured from Lewis rats: amniotic fluid-derived mesenchymal stem cells (AF-MSCs), bone marrow-derived MSC (BM-MSCs), amniotic fluid-derived neural stem cells (AF-NSCs), and neonatal enteric NSC (E-NSCs) [3]. These specific stem cell lines were chosen because we have previously demonstrated their individual efficacy in treating experimental NEC. [3,4] In addition, we have demonstrated that different

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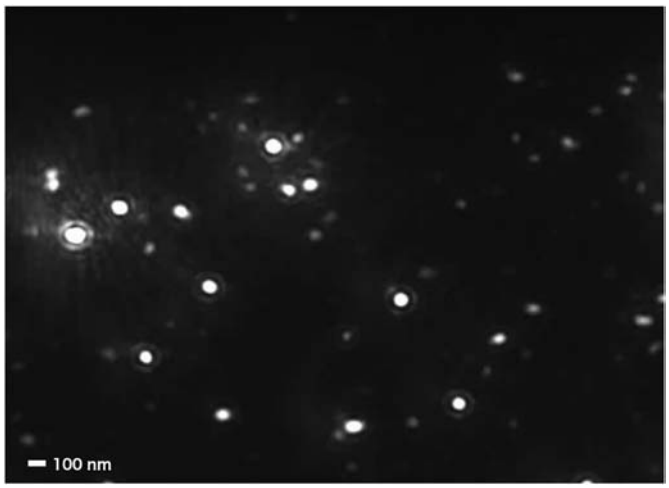

Fig. 1. Representative NanoSight image of purified exosomes. The exosomes shown were purified from AF-MSC. Similar results were seen with exosomes purified from the other three types of SC. Scale bar = 100 nm.

types of stem cells target different tissues. For example, NSCs target the injured enteric nervous system in our experimental NEC model, and improve intestinal motility after NEC. Because of these different effects, we wanted to investigate whether exosomes derived from these different types of stem cells might function differently from one another in protection of the intestines from NEC.

All stem cell lines were confirmed by flow cytometry for specific cell surface markers consistent with the different stem cell lines. AF-MSC cells were positive for CD29 (ThermoFisher, Waltham, MA), CD49e (ThermoFisher), CD90 (ThermoFisher), and Oct4 (Novus Biologicals, Littleton, CO), which are markers of MSCs and cells derived from AF. These cells were also negative for the hematopoietic precursor cell markers CD11 (ThermoFisher) and CD45 (ThermoFisher), as expected. BM-MSCs were positive for CD90, confirming their identity as MSC, and negative for CD11 and CD45. Both NSC cell populations were confirmed to be positive for Nestin (R&D Systems, Minneapolis, MN), a transient intermediate filament protein only expressed in NSCs and not in mature neural tissue.

Additionally, both mesenchymal stem cell lines were confirmed to be multipotent by differentiation along adipocytic and osteocytic cell lines using commercially available differentiation kits (StemPro Adipogenesis Differentiation Kit, and StemPro Osteogenesis Differentiation Kit, ThermoFisher).

1.2. Exosome generation and collection

Exosomes were generated and collected based upon published methods [9,11]. To generate exosomes from MSC populations, which grow in an adherent fashion in cell culture flasks, SC growth-supporting medium was replaced with media lacking fetal bovine serum (FBS). This exosome generation media was composed of Minimum Essential Medium Alpha with GlutaMAX™ (MEM- α, ThermoFisher) and 1% penicillin/streptomycin/amphotericin B (PSA, ThermoFisher).

Exosomes were generated from NSC populations by placement of SC into media lacking SC-supportive growth factors (EGF, FGF). Generation media were composed of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/ F12, ThermoFisher) supplemented with 4% chicken embryo extract (Gemini Bio-Products, West Sacramento, CA), 2% PSA, and 1× N-2 supplement (ThermoFisher). Cells in culture were

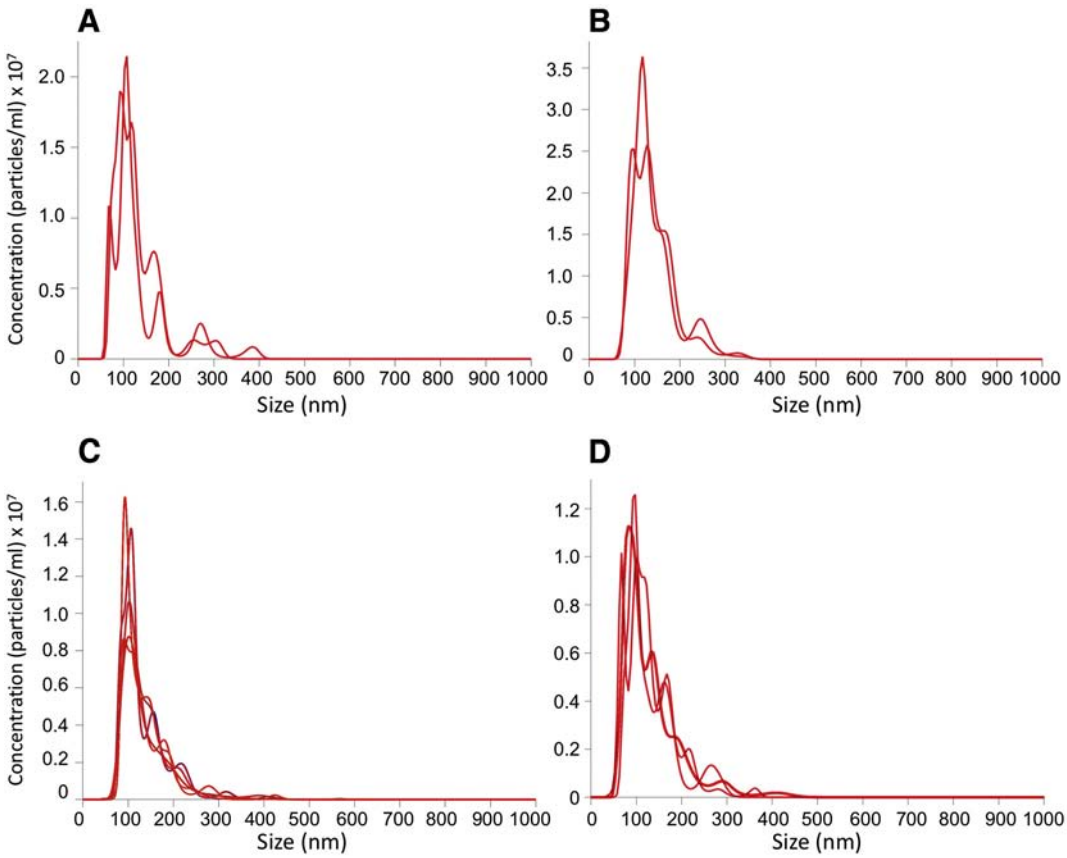

Fig. 2. Representative plots of particle size in exosome suspensions. The multiple lines represent multiple analytic runs of a single sample solution, which were then averaged. The exosomes analyzed were from: (A) AF-MSC; (B) BM-MSC; (C) AF-NSC; (D) E-NSC.