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PREVIEWS

Previews highlight research articles published in the current issue of STEM CELLS TRANSLATIONAL MEDICINE, putting the results in context for readers.

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Mouse epiblast stem cells (EpiSCs) are derived from the postimplantation epiblast of the developing embryo and resemble human embryonic stem cells (ESCs) in terms of morphology, the pathways employed for self-renewal, and other related molecular characteristics.[1,2] Mouse EpiSCs exist in a "primed" pluripotent state and are epigenetically distinct from mouse ESCs, which themselves exist in a "naïve" pluripotent state (or the ground state).[3] Detailed analyses of the conversion of EpiSCs into the naïve pluripotent state have fostered an in-depth understanding of the intricate reprogramming mechanisms involved, research which has supported the successful derivation of human naïve ESCs. Mouse EpiSCs maintain their self-renewal capacity via signaling through the Activin/Nodal and fibroblast growth factor (FGF)/ERK pathways; furthermore, Wnt signaling pathway inhibition can favor EpiSC growth and pluripotency.[4] EpiSCs also possess the potential to differentiate into cells of the three germ-layers, can form teratomas, and undergo random X-chromosome inactivation like the postimplantation epiblast.[3] Overall, EpiSCs represent a valuable tool for the understanding of critical developmental mechanisms. In the first of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Gao et al employ a massive mutagenesis protocol in their newly developed mouse haploid EpiSCs to identify candidate genes that modulate the reprogramming of EpiSCs to naïve pluripotency.[5] In a Related Article published recently in *STEM CELLS*, Sudheer et al highlighted the important role of FGF signaling in the differentiation of mouse ESCs through an EpiSC-like state into distinct presomitic mesodermal cell types in a study that may permit a deeper understanding of musculoskeletal disorders that arise during early development.[6]

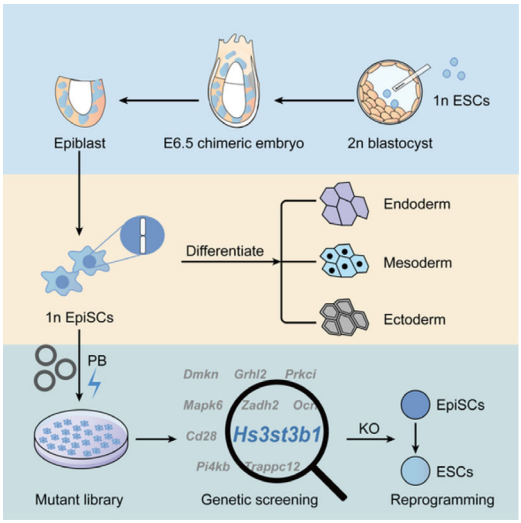
Bacterial and viral pneumonia and sepsis represent the most common causes of acute respiratory distress syndrome (ARDS), which remains a leading cause of disability and death in critically ill patients.[7] Excessive pulmonary inflammation mediated by the reaction of resident macrophages to infection represents the main characteristic of ARDS pathophysiology.[8] Although the search for effective treatments for ARDS has been ongoing for many years, this area has recently come under sharp focus due to the worldwide impact of coronavirus disease 2019 (COVID-19), a pneumonia-like disease caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[9,10] Severe COVID-19 patients present with hyper-inflammation, an overactive immune response that triggers a "cytokine storm," and a prothrombotic state that can progress to ARDS development, with reported patient mortality reaching over 50%.[11] Mesenchymal stem cell (MSC) therapy represents a potentially effective treatment option for ARDS, with ongoing trials now evaluating this approach in severe COVID-19 patients and related research attempting to delineate the molecular mechanisms behind the therapeutic effect of MSCs in the bacterially-induced form of the disease. In the second of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Lanzoni et al report on the encouraging findings of a double-blind, randomized, controlled, early phase clinical trial of umbilical cord-derived MSC treatment in patients with COVID-19-related ARDS.[12] In a Related Article published recently in *STEM CELLS*, Jackson et al demonstrated that mitochondrial transfer to macrophages represents a crucial mechanism by which MSCs mediate their therapeutic capacity in bacterially-induced ARDS.[13]

FEATURED ARTICLES

Haploid Epiblast Stem Cell-Based Screen Highlights Modulators of Naïve Reprogramming

Haploid EpiSCs, which contain just one set of chromosomes, could provide a powerful platform to study a variety of biological processes through genetic screening; however, attempts to derive such cells from postimplantation embryos have failed, mainly due to the severe diploidization observed during differentiation.[14,15] In the hope of solving this vexing problem, researchers led by Luyuan Li, Yan Liu, and Ling Shuai (Nankai University, Tianjin, China) recently sought to generate haploid EpiSCs with the help of a p53 knockout[16] and then apply them to genetic screening to delineate the mechanisms involved in mammalian reprogramming. As reported in *STEM CELLS Translational Medicine*,[5] Gao et al generated haploid EpiSCs from postimplantation chimeric mouse embryos at embryonic day 6.5, discovering that they shared molecular characteristics with wild-type diploid EpiSCs and possessed the expected primed-state morphology. Importantly, these EpiSCs also maintained their haploid nature,

expressed EpiSC-specific genes, possessed a stable genome, and exhibited the potential to differentiate into cells of the three germ layers, which encouraged the authors to explore their utility in genetic screening approaches. Employing a massive mutagenesis protocol, they screened for candidates that inhibit reprogramming of EpiSCs to the naïve state (like mouse ESCs) and validated Hst3st3b1, a gene that encodes heparan sulfate glucosamine 3-O-sulfotransferase 3B1 (a key component in generating heparan sulfate fine structures involved in diverse biologic activities), as a prime candidate. Overall, this exciting study provides evidence for the utility of haploid EpiSCs in research into fundamental biological processes.



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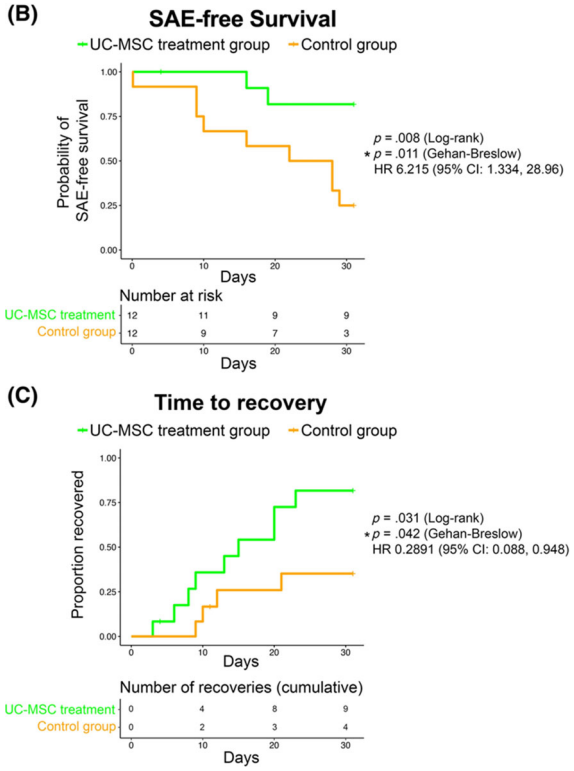
Trialing Umbilical Cord-MSCs as a Treatment for COVID-19-Related ARDS

Patients who develop severe cases of COVID-19, a pneumonia-like disease caused by SARS-CoV-2 virus infection, can often progress to ARDS. A reported mortality in patients with COVID-19 and ARDS of over 50%[11] highlights the dire need for novel therapies that can attenuate the excessive inflammatory response, accelerate the recovery of functional lung tissue, and reduce mortality rates. Researchers led by Camillo Ricordi (University of Miami, Florida) recently reported the results at one month of follow-up of a double-blind, phase I/IIa, randomized, controlled trial to establish safety and explore the efficacy of allogeneic umbilical cord-derived (UC-) MSC infusions[17,18] in hospitalized patients with ARDS secondary to COVID-19. As reported in *STEM CELLS Translational Medicine*,[12] Lanzoni et al evaluated 24 patients randomized to a UC-MSC group, who received two intravenous infusions of 100 million UC-MSCs 72 hours apart, or a control vehicle-treated group. The trial failed to observe any adverse events or serious adverse events associated with UC-MSC infusions, suggesting the safety of this approach. Furthermore, exploratory efficacy analyses suggested that UC-MSC infusions associated with a decrease in pro-inflammatory cytokines linked to COVID-19-related "cytokine storm" and a significant improvement in patient survival, serious adverse effect-free survival, and time to recovery. Overall, these highly encouraging findings support larger trials to estimate and establish the efficacy of UC-MSC-based therapy for severe COVID-19 patients suffering from ARDS.



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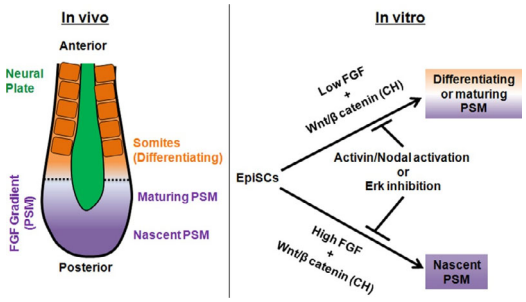
RELATED ARTICLES

Exploring the Influence of FGF on ESC Differentiation into Presomitic Mesoderm

The presomitic mesoderm (PSM) of the developing embryo represents the precursor of the segmental somites that eventually give rise to the axial skeleton and skeletal muscles, tendons, dorsal dermis, and adipose tissue.[19,20] A recent *STEM CELLS* article by researchers led by Smita Sudheer and Bernhard G. Herrmann (Max Planck Institute for Molecular Genetics, Berlin, Germany) reported a protocol for the efficient induction of PSM from mouse ESCs after passing through an EpiSC-like state that was developed with the help of mesodermal/

presomitic mesoderm marker gene reporters.[6] The activation of Wnt signaling by glycogen synthase kinase-3 inhibition (via CHIR99021 or "CH" treatment) prompted the formation of EpiSC-like cells (primed pluripotency) from ground state ESCs (naïve pluripotency), with FGF signaling then promoting the high-efficiency induction of PSM; however, the authors discovered that altering FGF concentrations altered the state of the PSM cells. While high FGF concentrations supported posterior (nascent) PSM formation, low FGF concentrations generated anterior (maturing/differentiating) PSM, in agreement with previous in vivo findings. Furthermore, the study discovered that Activin/Nodal inhibition enhanced Wnt/FGF signaling to promote the induction of PSM. Overall, the team behind this exciting research hoped that their findings would support further research employing the directed differentiation of ESCs into the gene regulatory networks that control PSM formation and differentiation, which may ultimately foster a deeper understanding of musculoskeletal disorders that arise during early development.



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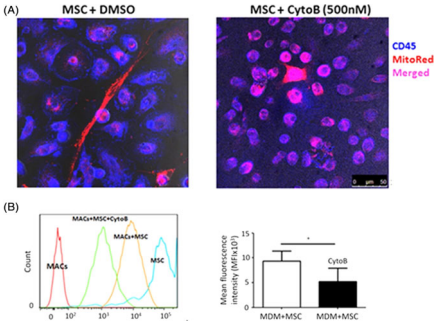
Defining the Mechanisms Involved in MSC Therapy for ARDS

Researchers led by Anna Krasnodembskaya (Queen's University of Belfast, UK) previously demonstrated how the secretion of antimicrobial factors[21] and the modulation of phagocytic capacity of host monocytes[22] and alveolar macrophages[23] mediated the antimicrobial effect of MSCs in ARDS models. Due to the increasing body of evidence suggesting the overall importance of direct cell contact between MSCs and target cells to therapeutic outcomes, the Krasnodembskaya lab recently explored the influence of mitochondrial transfer via tunneling nanotubes on bacterial clearance by innate immune cells following MSC therapy. As reported in their recent *STEM CELLS* article,[13] Jackson et al first employed an in vivo model of *Escherichia coli*-induced pneumonia to demonstrate how MSC therapy failed in the absence of alveolar macrophages (induced by intranasal liposomal clodronate administration), and that mouse macrophages and human monocyte-derived macrophages displayed enhanced bioenergetic and phagocytic activity in the presence of MSCs. Excitingly, fluorescent imaging and flow cytometry then highlighted the importance of cell-to-cell contact by linking the tunneling nanotube-mediated transfer of mitochondria from MSCs to macrophages to these significantly improved activities. The authors underscored the overall importance of tunneling nanotubes by demonstrating how the inhibition of mitochondrial transfer from MSCs to macrophages by blocking tunneling nanotube formation (via Cytochalasin B or "CytoB" treatment) completely inhibited phagocytosis by macrophages and antimicrobial effect of administered MSCs. Overall, this fascinating study described a novel mechanism mediating the therapeutic effects of MSC administration in bacterially-induced ARDS.



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