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Mesenchymal Stromal Cell Extracellular Vesicles: A New Approach for Preventing Bronchopulmonary Dysplasia?

Bronchopulmonary dysplasia (BPD) is the most common complication of prematurity, affecting about one-third of infants born less than 1.5 kg and less than 29 weeks gestational age (1). BPD is characterized by abnormal bronchial and bronchiolar mucosal metaplasia and hyperplasia, decreased number of alveoli, and abnormal vascular organization, leading to a chronic disease that affects the lung parenchyma, pulmonary circulation, and brain development.

Lung formation begins between weeks 3 and 6 of gestation, but the maturation of peripheral lung saccules into mature alveoli does not occur until the last trimester (28–40 wk). Thus, preterm infants are often forced to breathe at a time before alveolar differentiation and vascularization are complete. Lacking sufficient respiratory capacity, many premature infants are subjected to recurrent lung injury from the high concentrations of supplemental oxygen and mechanical ventilation that are needed to keep them alive. Although most infants with BDP survive, the majority are left with a chronic lung disease characterized by decreased alveolarization, cystic emphysema, fibrosis, and pulmonary vascular remodeling (2). Pulmonary hypertension develops in nearly 60% of severe BPD infants by Day 7, and its presence beyond 3 months is associated with mortality rates of 40–50% (3). Neurocognitive development is also impaired in BPD, likely due to prematurity, hypoxia, and systemic inflammation, leading to a greater frequency of cerebral palsy, intellectual disability, and reduced intelligence quotient scores in very-low birth weight infants who develop BPD than in those who do not (4).

Inflammation induced by pneumonia, systemic nosocomial infection, and barotrauma plays a major pathogenic role. Concentrations of neutrophils and macrophages that produce proteases, reactive oxygen species, and inflammatory cytokines, including IL-1β, IL-6, and IL-8, are elevated in tracheal aspirates of BPD infants (5). Interestingly, infants with respiratory distress syndrome who progress to BPD exhibit persistently elevated concentrations of inflammatory cells and cytokines, whereas those who do not develop BPD show a marked decrease within the first 1–2 weeks (5).

Despite major advances in perinatal care, no specific therapies for BPD have been found to be effective, and treatment is directed at minimizing further lung damage and supporting normal lung development. Advancing treatment of BPD will require therapies that combat both the arrested development and persistent inflammation that occurs. One such approach has been the use of mesenchymal stem cells (MSCs). These pluripotent cells typically transform into osteoblasts, adipocytes, and chondroblasts *in vitro*. However, their limited ability to differentiate into nonmesodermal cells *in vivo* challenges their identity as true stem cells; thus, the more current term is mesenchymal stromal cells. MSCs have been shown to differentiate into surfactant-producing epithelial cells both *in vitro* and after injection into live animals (6). In addition to their

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regenerative properties, MSCs have strong immunomodulatory effects, including the ability to shift macrophage activation from a pro- to an antiinflammatory phenotype. They also inhibit T-cell and B-cell proliferation and favor the generation of regulatory B cells and regulatory T cells, the latter of which has been found to be decreased in patients with BPD (7).

Many biologic effects of MSCs are mediated by the extracellular vesicles (EVs) they secrete. EVs are important mediators of cell-to-cell communication and have increasingly been shown to recapitulate beneficial properties of MSCs without the potential adverse effects of whole-cell therapy. A growing body of literature supports their efficacy in animal models of lung injury and pulmonary hypertension. Beneficial effects of MSC EVs have been reported in preclinical pneumonia models of adult acute respiratory distress syndrome in mice as well as in an *ex vivo* perfused human lung model (8, 9). In these studies, MSC EVs reduced inflammation, improved lung fluid and protein balance, and enhanced lung repair. Similarly, MSC EVs have been shown to prevent and reverse pulmonary hypertension in multiple animal models where they increase the number of peripheral pulmonary vessels and the number of antiinflammatory macrophages (10). Considering the prominent roles that lung injury and pulmonary vascular remodeling play in the pathogenesis of BPD, MSC EVs may have unique advantages over other immunosuppressive therapies for this disease. Indeed, several labs have used this approach to attenuate lung injury in rodent models of BPD (11, 12). The mechanisms responsible for their beneficial effects are unclear but likely include the transfer of RNA, intracellular proteins, and mitochondria to different cell types, including alveolar epithelial cells, alveolar macrophages, and bone marrow progenitor cells (13). Recently, intravenously administered MSC EVs were shown to interact with myeloid cells in the lung, where they suppressed pro-inflammatory macrophage activation and promoted an immunosuppressive monocyte phenotype resulting in restored alveolar architecture, blunted fibrosis, and reduced pulmonary vascular remodeling (12).

Although the results of earlier studies have been encouraging, it is not clear if findings obtained primarily from the hyperoxia murine model of BPD can translate to human disease and if the attenuation of lung injury and vascular injury translates into improved neurodevelopment. In this issue of the *Journal*, Lithopoulos and colleagues (pp. 1186–1201) tested the effects of MSC EVs in an animal model of BPD that incorporated key components associated with disease pathogenesis in human infants: mechanical ventilation, inflammation, and supplemental oxygen (14). Postnatal mice were injured with intraperitoneal endotoxin followed by mechanical ventilation with 40% oxygen. In addition to alveolar and vascular rarefaction mimicking BPD, the authors were able to demonstrate impairments in neural progenitor cell function, including reduced neurosphere and oligodendrocyte formation. Intratracheal administration of MSC EVs before ventilation resulted in reduced inflammation and a substantial improvement in lung architecture and vessel formation. Importantly, MSC EVs also induced significant improvement in neuroprogenitor cell function. Whether the latter occurred as a direct effect of MSC EVs on neuronal cells or an indirect effect caused by the reduction in lung injury and inflammation is unclear. However, the authors were able to demonstrate that MSC EVs injected intratracheally were taken up by brain tissue and improved neurosphere formation *in vitro*. Together, the findings suggest that MSC EVs have the ability to mitigate many devastating effects of BPD and may represent a major advance in the management of this disease. Their findings are limited by the lack of functional outcomes such as the effects of MSC EVs on lung compliance, airway obstruction, pulmonary hemodynamics, and neurocognitive function. Additional studies demonstrating that improvements in lung architecture, vascular remodeling, and neuroprogenitor cell function translate into healthier adult animals with better pulmonary function and cognitive skills are needed if MSC EVs are to be developed for clinical use. Other hurdles must also be overcome, including the creation of better technologies to produce the large number of EVs needed while limiting variation in particle size and content. There are also likely to be regulatory issues related to the consistency of the clinical product, its shelf-life, and minimally effective dose and dosing interval. Despite these limitations, nearly a dozen clinical studies evaluating MSC as a treatment for BPD have been organized, and efforts are ongoing to initiate safety and feasibility trials using MSC EVs. Although one study was halted due to business decisions involving product development (ClinicalTrials.gov Identifier: NCT03857841), 3 infants received the MSC EV preparation, underscoring how close the field has come to clinical trials. ■

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Can Diagnostic Specificity and Phenotyping Aid in Evaluating Cardiometabolic Risk of Maternal Sleep-disordered Breathing?

Sleep-disordered breathing (SDB) is a common disorder in pregnancy but its prevalence varies with the degree of risk in the population, methodology, and definition (1, 2). Further, associations between SDB in pregnancy and adverse outcomes are well documented. Large population-based studies have demonstrated an association with hypertensive disorders of pregnancy, gestational diabetes, and severe maternal morbidity (3, 4). Moreover, the prospective nuMoM2B (Nulliparous Pregnancy Outcomes Study Monitoring Mothers-to-Be) study confirmed the links between SDB and preeclampsia and gestational diabetes (1). Indeed, after correcting for maternal age, body mass index, chronic hypertension, and gestational weight gain, SDB in early- and mid-pregnancy was associated with a twofold increase in the risk of preeclampsia and a nearly threefold increase in the risk of gestational diabetes (1). These adverse outcomes are important causes or key contributors to severe maternal morbidity and maternal mortality, are the source of an important societal and financial burden, and have been linked to long-term adverse outcomes for mother and offspring.

Mouse models of preeclampsia show subsequent cardiac abnormalities, including higher fibrin deposit counts in cardiomyocytes as well as functional abnormalities as measured by ultrasonography before and after dobutamine stress tests (5). Women with a history of preeclampsia have higher myocardial mass and thicker left ventricular walls at 12-year follow-up, abnormalities that correlate with pregnancy antiangiogenic profiles (6). A recent meta-analysis confirmed (7) that women with preeclampsia have a higher risk of major cardiac events and cardiovascular disease. Population-based studies demonstrate that in middle-aged women undergoing coronary revascularization, a single episode of maternal placental syndrome doubles the risk of death, whereas recurrent maternal placental syndrome quadruples that risk (8). Similarly, gestational diabetes is an established risk factor for the development of type II diabetes (9) and cardiovascular disease in women (10). Despite the contribution of SDB to adverse cardiovascular outcomes in the general population and its link to preeclampsia and gestational diabetes, little is known about the cardiovascular and metabolic risks of maternal SDB after delivery.

In this issue of the *Journal*, Facco and colleagues (pp. 1202–1213) note the nuMoM2B Heart Health study followed a subset of the cohort ($n = 1{,}964$) who had a level III home sleep test either in early or late gestation (or both) of their first pregnancy and assessed for new-onset hypertension and metabolic syndrome 2–7 years after the index pregnancy (11). The study used two definitions for SDB: apnea–hypopnea index (AHI) of $\geqslant$5 events/h (3% desaturation rule for hypopnea), and oxygen desaturation index (ODI) of $\geqslant$5 events/h. The primary analyses used dichotomous AHI and ODI definitions from the early- or mid-pregnancy assessments to examine the risk of incident hypertension and metabolic syndrome (three or more of the following: elevated waist circumference, triglycerides, glucose, and/or blood pressure and/or reduced high-density lipoprotein concentrations) after a median of ~3 years. The secondary analyses examined cross-sectional associations between AHI and ODI at the 2- to 7-year follow-up and the same cardiometabolic outcomes. The investigators also assessed the trajectory of SDB status between pregnancy and follow-up and its link to these outcomes. There was no significant association between SDB in pregnancy and incident hypertension when SDB was defined by AHI criteria, but when SDB was defined

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