The preterm lungs and perinatal stress

IMPACT

Preterm birth together with perinatal stress, including pre- and postnatal inflammatory and injurious exposure (1), frequently result in complications after birth and the development of chronic lung disease (CLD) in preterm neonates (2, 3). Hospitalization and re-hospitalization rates for children with CLD are extremely high, especially in the first years of life and are linked to enormous health care costs (around 740.000 Euro per CLD-affected neonate in the first year) (4, 5).

Due to incomplete understanding of neonatal morbidities and the lack of efficient clinical management, neonatal CLDs have great impact on the continuous lung development of a child and significantly increase the risk for respiratory problems in later childhood and even in adulthood.

In this context, prenatal inflammation has been strongly associated with the exacerbation of postnatal injury by affecting the preterm lungs already prenatally (4-6). Considering that endogenous stem/progenitor cells are crucially involved in fetal lung development, the first aim of this thesis was to gain more insight into the impact of prenatal stress on endogenous epithelial stem/progenitor populations in the developing lungs.

Secondly, current interventions are primarily used to ease symptoms, but they do not prevent postnatal injury and have no long-lasting therapeutic effects, resulting in long term respiratory problems (6). Due to their strong immunomodulatory and regenerative characteristics, bone-marrow derived multipotent adult progenitor cells (MAPC) in particular, have been postulated to be a promising therapeutic option for preterm neonates (7). With the aim to prevent or reduce postnatal injury in preterm lambs we administered MAPCs earlier than conventional treatment, during a series of perinatal stressors.

In our prenatal inflammation model (chapter 2 and 3) we showed that *in utero*-induced inflammation negatively affected endogenous epithelial stem/progenitor populations in the preterm ovine lungs. Additionally, prenatal inflammation affected the preterm lungs beyond preterm birth and sensitized the preterm lungs to postnatal adverse outcomes (chapter 4). These studies are clinically highly relevant as they emphasize the crucial role of inflammatory stress during prenatal lung development because it strongly affects important developmental factors already *in utero*. Considering the essential role of endogenous stem/progenitor cells during lung development, prenatal disturbances in these cell populations might lead to irreversible changes in lung development. This could explain why the preterm lungs in children with CLD have difficulties to recover from perinatal stress and why they are prone to CLD in adulthood. Not only in the preterm lungs impeded development has been reported after perinatal stress, but also in other organ systems.

With their fundamental role in all developing organs, the investigation of endogenous stem/progenitor cells should therefore be expanded to other preterm and compromised organs, which would also help to determine possible organ-spanning effects. In the gastrointestinal system of infants and mice with necrotizing enterocolitis, disturbances in

stem cell renewal and altered activity in Wnt/b-catenin pathways have been measured, and resulted in decreased intestinal renewal (8). Interestingly, Wnt pathway alterations have also been reported in prenatal stress-exposed lungs (9). It is tempting to speculate that there could be a link between gastrointestinal and lung injury, in particular when endogenous stem/progenitor cells are disturbed in both organs by perinatal stress. Crosstalk between organs is a common phenomenon and an impacted gastrointestinal tract, e.g. by antibiotics, has been shown to predispose to the development of asthma, especially when antibiotics were administered shortly after birth (10). Prenatal inflammation-induced injury in one preterm organ might enhance the adverse development of another organ by endocrine signaling, together with the ongoing peripheral inflammatory response (11). More studies are needed to determine endogenous stem/progenitor cell changes in preterm organs and to elucidate potential crosstalk between organs with altered endogenous stem/progenitor populations and to study their contribution to postnatal adverse organ development.
These prenatal insights should be used to reevaluate current clinical management of preterm infants, suggesting that the focus of clinical diagnosis and treatment should be shifted towards more awareness for prenatal stress and prenatal fetal maldevelopment. For instance, improving *in utero* monitoring of mother and child for signs of infection, e.g. by advanced sampling techniques for the unborn fetus (amniotic fluid, cord blood), could enable an earlier identification of silent infections, such as chorioamnionitis. Particularly, early detection of prenatal inflammation by amniotic fluid analysis has been shown to effectively ameliorate the clinical management of predicted chorioamnionitis, when incorporated in the clinical assessments (12). In addition, pre-clinical studies in ovine chorioamnionitis models indicate that clinical decision-making for both mother and neonate can significantly be improved when results of volatile organic compound (VOC) testing as a first line point-of-care-test are taken into account. Notably, VOC testing is also a safer and less invasive option than amniotic fluid analysis (13, 14).
Recognizing prenatal changes as the starting point of adverse pulmonary development will also help to create early prediction models during pregnancy (15, 16). While current prediction models focus on postnatal risk factors to establish an estimate for CLD development, the occurrence of prenatal stress has been neglected to this point. Taking pre- and postnatal risk factors into account will result in a more precise determination of the risk of postnatal adverse outcomes.

Understanding the impact of prenatal stress on fetal development will also encourage the development of efficient therapeutics to address and overcome prenatal disturbances. In this context we demonstrated in **chapter 4** that early administration of MAPCs in the course of perinatal stress prevented functional respiratory problems. Evidence was provided that enhanced regeneration and immune modulatory processes (at least in part) prevented the

adverse functional pulmonary outcomes following perinatal inflammation. Currently, research focusses on refinement of treatment strategies with MAPCs and other MSC subtypes, to urge the implementation of MSCs/MAPCs as neonatal treatment, especially for preterm infants. Our stem cell therapy study described in chapter 4 fits elegantly within this regime aspect and the results indicate that timing of MAPC treatment matters. More precisely, our findings indicate that administration of stem cells relatively short before or during the onset of a perinatal inflammatory hit, is an essential and potent strategy in preventing postnatal adverse pulmonary outcomes. To broaden our understanding of therapeutic effects of MAPCs, also the outcome on other organ systems needs to be evaluated. Paracrine-mediated effects of MAPC should be further investigated to identify certain organ-spanning and organ-specific effects, such as increased regenerative properties, as we have observed in the preterm lungs after perinatal stress and prenatal MAPC treatment.

MSC/MAPC investigations also focus on increasing the effectiveness and efficiency of stem cell treatment. One method hereby is the *in vitro* pre-condition of stem cells to prime them to exert specific effects once introduced into a recipient. This process, called licensing, can potentially increase the pharmacological effects of stem cells, which reduces the overall number of stem cells needed for therapy (17-19).

Notably, MAPCs known as MultiStem®Cells, are off-the-shelf therapeutics, FDA approved (20), and already under investigation in clinical phase 2 and 3 studies for adult with stroke (21-23). With regard to respiratory diseases, ongoing studies on therapeutic effects of MAPCs in patients with acute respiratory distress syndrome show first promising preliminary findings (24). Considering the fast progression in cell-based therapy, further clinical studies, such as those from Chang *et al.*, will most likely soon also result in the first commercially available therapeutic for preterm infants (25-26).

In this thesis, we showed that prenatal stress has a great impact on fetal development and that early MAPC treatment can prevent immediate postnatal adverse outcomes, but yet, at this point we cannot predict whether prenatal changes will persist postnatally and throughout childhood and whether MAPC have a long lasting effect on the immature lungs. To address the issue of missing long-term data, our group established a pre-clinical longitudinal ovine study, in which lambs are exposed to perinatal stress (as in chapter 4) while their development is followed up to one year of life, which is comparable with an young adult in humans. This model is designed as a follow-up for perinatal stress in preterm lungs, to study the pathological long-term consequences and the potentially increased risk to childhood CLDs, such as asthma and wheezing, and the predisposition to chronic lung disease as COPD in adults, which all have been associated with perinatal stress during birth. This model give us the unique opportunity to study lung development and lung pathologies with clinically relevant functional and structural tools beyond birth and throughout the late

neonatal period, childhood and young adulthood. The acquired knowledge will be verified in human cohort studies and vice versa.

On the other hand, this longitudinal model can be used to study therapeutic benefits of MAPC treatment throughout lung development and will elucidate whether MAPC treatment can prevent or reduce the predisposition to CLD in child- and adulthood.

Increasing our understanding of adverse neonatal development and establishing appropriate efficient therapies will likely reduce the incidence of children with CLD and reduce the risk for long-term respiratory problems. Addressing adverse outcomes in neonates from the developmental and therapeutic perspective will ultimately result in a better start after birth and improve patient outcomes in a long-term manner. These advances will also have effects on the social and economic level, for instance by decreasing the emotional and financial stress for families. Health care systems will potentially experience less (re-) hospitalization while hospital visits might be significantly reduced in their duration.

Altogether, this thesis delivers proof that prenatal inflammation impacts fetal development by affecting their endogenous stem/progenitor cells, which potentially can be responsible for adverse development postnatally. At the same time, we show that early administration of cell-based treatment can prevent postnatal adverse outcomes. Early identification of prenatal disturbances and early treatment of developmental changes appear essential in preterm infants because it potentially reduces postnatal injury, decreases the risk for long lasting respiratory problems in child- and adulthood, and enables infants a better start into the future.

REFERENCES

1. Wright RJ. Perinatal stress and early life programming of lung structure and function. Biol Psychol. 2010;84(1):46-56.
2. Bhandari A, Carroll C, Bhandari V. BPD Following Preterm Birth: A Model for Chronic Lung Disease and a Substrate for ARDS in Childhood. Front Pediatr. 2016;4:60.
3. Thebaud B. Stem Cells for Extreme Prematurity. Am J Perinatol. 2019;36(S 02):S68-S73.
4. Jacob J, Lehne M, Mischker A, Klinger N, Zickermann C, Walker J. Cost effects of preterm birth: a comparison of health care costs associated with early preterm, late preterm, and full-term birth in the first 3 years after birth. Eur J Health Econ. 2017;18(8):1041-6.
5. Korvenranta E, Lehtonen L, Rautava L, Häkkinen U, Andersson S, Gissler M, et al. Impact of very preterm birth on health care costs at five years of age. Pediatrics. 2010;125(5):e1109-e14.
6. Um-Bergström P, Hallberg J, Thunqvist P, Berggren-Brostrom E, Anderson M, Adenfelt G, et al. Long term pulmonary outcome after mild to severe bronchopulmonary dysplasia. Children (Basel). 2016; 7(12): 283.
7. Collins A. Stem-cell therapy for bronchopulmonary dysplasia. Cur. Opin. Pediatr. 2020;32(2):210-5.
8. Li B, Lee C, Cadete M, Zhu H, Koike Y, Hock A, et al. Impaired Wnt/β-catenin pathway leads to dysfunction of intestinal regeneration during necrotizing enterocolitis. Cell Death Discov. 2019;10(10):1-11.
9. Kuypers E, Willems MG, Collins JJ, Wolfs TG, Nitsos I, Jane Pillow J, et al. Altered canonical Wingless-Int signaling in the ovine fetal lung after exposure to intra-amniotic lipopolysaccharide and antenatal betamethasone. Pediatr Res. 2014;75(2):281-7.
10. Ortqvist AK, Lundholm C, Kieler H, Ludvigsson JF, Fall T, Ye W, et al. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. BMJ. 2014;349:g6979.
11. Armutcu F. Organ crosstalk: the potent roles of inflammation and fibrotic changes in the course of organ interactions. Inflamm Res. 2019;68(10):825-39.
12. Gultekin-Elbir EE, Ford C, Genc MR. The value of amniotic fluid analysis in patients with suspected clinical chorioamnionitis. J Perinat Med. 2019;47(5):493-9.
13. Ophelders D, Boots AW, Hutten MC, Al-Nasiry S, Jellema RK, Spiller OB, et al. Screening of Chorioamnionitis Using Volatile Organic Compound Detection in Exhaled Breath: A Pre-clinical Proof of Concept Study. Front Pediatr. 2021;9:617906.
14. Goedicke-Fritz S, Werner T, Niemarkt HJ, Wolfs TG, Baumbach JI, Kemp MW, et al. Detection of Volatile Organic Compounds as Potential Novel Biomarkers for Chorioamnionitis–Proof of Experimental Models. Front Pediatr. 2021:747.
15. Valenzuela-Stutman D, Marshall G, Tapia JL, Mariani G, Bancalari A, Gonzalez A, et al. Bronchopulmonary dysplasia: risk prediction models for very-low- birth-weight infants. J Perinatol. 2019;39(9):1275-81.
16. Ding L, Wang H, Geng H, Cui N, Huang F, Zhu X, et al. Prediction of Bronchopulmonary Dysplasia in Preterm Infants Using Postnatal Risk Factors. Front Pediatr. 2020;8:349.
17. Dunbar H, Weiss DJ, Rolandsson Enes S, Laffey JG, English K. The Inflammatory Lung Microenvironment; a Key Mediator in MSC Licensing. Cells. 2021;10(11).
18. Szabo E, Fajka-Boja R, Kriston-Pal E, Hornung A, Makra I, Kudlik G, et al. Licensing by Inflammatory Cytokines Abolishes Heterogeneity of Immunosuppressive Function of Mesenchymal Stem Cell Population. Stem Cells Dev. 2015;24(18):2171-80.
19. Silva-Carvalho AÉ, Sousa MRR, Alencar-Silva T, Carvalho JL, Saldanha-Araujo FJC, reviews gf. Mesenchymal stem cells immunomodulation: The road to IFN-γ licensing and the path ahead. Cytokine Growth Factor Rev. 2019;47:32-42.
20. Khan RS, Newsome PN. A Comparison of Phenotypic and Functional Properties of Mesenchymal Stromal Cells and Multipotent Adult Progenitor Cells. Front Immunol. 2019;10:1952.

21. Yang B, Hamilton JA, Valenzuela KS, Bogaerts A, Xi X, Aronowski J, et al. Multipotent Adult Progenitor Cells Enhance Recovery After Stroke by Modulating the Immune Response from the Spleen. Stem Cells. 2017;35(5):1290-302.
22. Han D, Liu H, Gao Y, Feng J. Targeting Brain-spleen Crosstalk After Stroke: New Insights Into Stroke Pathology and Treatment. Curr Neuropharmacol. 2021;19(9):1590-605.
23. Athersys Inc. Clinical Pipeline [Internet]. Athersys Inc. [cited 2021]. Available from: https://www.athersys.com/clinical-trials/clinical-pipeline/default.aspx.
24. Athersys Inc. Acute Respiratory Distress Syndrome (ARDS) [Internet]. Athersys Inc. [cited 2021]. Available from: https://www.athersys.com/clinical-trials/ards/.
25. Chang YS, Ahn SY, Yoo HS, Sung SI, Choi SJ, Oh WI, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. J Pediatr. 2014;164(5):966-72 e6.
26. Wu X, Xia Y, Zhou O, Song Y, Zhang X, Tian D, et al. Allogeneic human umbilical cord-derived mesenchymal stem cells for severe bronchopulmonary dysplasia in children: study protocol for a randomized controlled trial (MSC-BPD trial). Trials. 2020;21(1):125.