Mesenchymal stromal cells as a tool to unravel the developmental origins of disease

Pia Todtenhaupt, 1,2 Melissa van Pel, 3,4 Arno A.W. Roest, 5 and Bastiaan T. Heijmans 6

The intrauterine environment can induce alterations of the epigenome that have a lasting impact on disease risk. Current human studies in the field focus on a single epigenetic mark, DNA methylation, measured in blood. For in-depth mechanistic insight into the developmental origins of disease, it will be crucial to consider innovative tissue types. Mesenchymal stromal cells (MSCs) may serve as a novel tool to investigate the full epigenome beyond DNA methylation, to explore other omics levels, and to perform functional assays. Moreover, MSCs can be differentiated into multiple cell types and thereby mimic otherwise inaccessible cell types. A first wave of studies supports the potential of MSCs and illustrates how the innovative use of this cell type may be incorporated in birth cohorts.

Unraveling the role of epigenetics in the developmental origins of disease

Epidemiologic studies have associated the intrauterine environment with disease susceptibility later in life [1–4]. Epigenetic (see Glossary) changes are considered a plausible molecular mechanism for this relationship [5–9]. Depending on the environment in utero, an embryo or fetus can acquire epigenetic changes at the level of DNA methylation or histone modifications in various tissues. This in turn will affect the potential of a gene to become expressed by regulating the accessibility of the DNA to the cellular transcription machinery and by recruiting or repelling transcription factors and chromatin modifiers [2,10]. The altered epigenetic configuration of a cell can be mitotically heritable and persistent throughout life [2]. Therefore, epigenetic mechanisms may confer the memory that is the cellular mechanism to explain how the intrauterine environment influences disease susceptibility up to decades later in life.

Indeed, prenatal exposure to maternal undernutrition, obesity, or chemicals such as those released by smoking are associated with differences in DNA methylation in humans [6,11,12]. In the setting of the Dutch famine, genome-wide analysis identified differences in DNA methylation between individuals prenatally exposed to severe malnutrition as compared with unexposed same-sex siblings, in particular for early gestational exposure [13–15]. Similarly, seasonal differences in The Gambia – where the rainy (‘hungry’) and dry (‘harvest’) season generates a natural variation in nutrient access – were associated with persistent epigenetic differences at the level of DNA methylation [7,8]. Studies of birth cohorts are increasingly reporting associations between prenatal exposures and DNA methylation differences in birth and childhood. For example, meta-analyses by the Pregnancy And Childhood Epigenetics (PACE) Consortium revealed DNA methylation profiles at birth in relation to maternal prepregnancy body mass index (BMI) and smoking [9,11,12]. Hence, the study of epigenetic mechanisms has great potential for unraveling the processes that explain the developmental origins of health and disease (DOHaD).

While birth cohorts and consortia hold advantages, including their large sample size and thus statistical power, they commonly rely on the study of peripheral tissues like whole (cord) blood, with a limited possibility for in-depth analysis. To advance the field, it is crucial to consider the

Highlights

The intrauterine environment can influence health throughout life. Although epigenetic changes are a plausible underlying mechanism, the specific molecular pathways remain largely undefined.

To advance mechanistic insight in the developmental origins of disease, innovative approaches are required to complement large-scale studies based on measurement of DNA methylation in blood.

Mesenchymal stromal cells (MSCs) are multipotent, easily accessible cells that facilitate a large variety of assays, enabling a new generation of studies in humans.

Although biobanking MSCs is more elaborate than freezing whole blood, it enables charting a comprehensive omics spectrum beyond DNA methylation, performing functional assays, and mimicking inaccessible tissues by differentiation.

Recent smaller-scale studies utilizing MSCs to investigate the developmental origins of disease demonstrated their potential to unravel the underlying mechanisms.
addition of innovative tissue types that will allow us to investigate a broader range of epigenetic mechanisms, chart the consequences on cell function, and explore tissue-specific effects. In this review, we outline the limitations in the field with the biological materials currently used, and go on to illustrate that MSCs isolated at birth have the potential to deliver in-depth insights into the epigenetic and functional mechanisms underlying the developmental origins of human disease.

Limitations of the commonly studied biological materials

The type of biological material and their storage conditions in epidemiological studies determine the epigenetic and functional aspects which can be assayed and how findings can be interpreted. This applies to both quasi-experimental settings [5–8] and birth cohorts [9,11,12], and has been noted previously [2,9,16]. In most of these studies, easily accessible but heterogenic peripheral tissues such as peripheral blood, cord blood, and/or buccal swabs were collected. Subsequent procedures for preparation and long-term sample storage – such as freezing and extraction of genomic DNA – are incompatible with maintaining viable cells and severely limit the possible assays that can be used. DNA isolated from frozen cells can be effectively used for the analysis of DNA methylation, as DNA methylation consists of a stable covalent addition of a methyl group to cytosine bases. By contrast, the binding of histones and other regulating proteins, such as transcription factors as well as the 3D structure of the chromatin, relies on noncovalent interactions that are lost during these isolation and freezing procedures.

While DNA methylation may control, stabilize, and reflect changes in gene expression, it ignores the fact that genomic regulation relies on the interplay between various molecular layers of the epigenome. This limits the functional interpretation of any observed epigenetic differences, as it is often not possible to examine effects on the transcriptome, proteome, and other downstream omics levels [9,17,18]. It is therefore not possible to assess the operative changes that may have been caused by an altered epigenetic regulation without retaining viable cells. Notably, animal experiments showed that prenatal nutrition status indeed has significant effects on the expression of transcription factors, histone modifications, miRNAs, and 3D chromatin interactions [19–23]. A second major limitation of the currently used biological materials is that the sampled tissues are heterogeneous and consist of multiple cell populations. As the epigenome’s primary role lies within development and cell differentiation, every cell type has its unique epigenome. When assaying DNA methylation of a heterogeneous cell population, such as peripheral blood, the measured methylation level for a specific CG dinucleotide is the mean methylation level of all the different cell types present in the sample. This means that an observed difference in DNA methylation between individuals with a contrasting exposure to prenatal factors may be derived from a relative difference in cell-type abundance. To account for such epigenetic differences, a range of approaches has been proposed, all relying on the correction in statistical models – such as predicting cell composition with reference panels, or correcting for cell-type composition using reference-free approaches – but they cannot account for the full complexity of cellular content [24–27]. Hence, the interpretation of observed epigenetic differences as actual differences, instead of being the consequence of changes in cell-type composition, remains debated. Moreover, the presence of different cell types leads to noise in epigenetic data, and cell-specific epigenetic changes will be diluted. Both factors may in part explain why reported differences in DNA methylation associated with prenatal exposures are generally modest, and there is no consensus as to the functional relevance of such differences observed in tissues containing different cell types.

The peripheral tissues assessed thus far often play no or only a limited role in the development of the disease phenotypes associated with exposures to adverse prenatal conditions. Data
indicating that epigenetic profiles in peripheral tissues mark those of target tissues remain inconclusive. Comparing multiple tissues from distinct embryonic origin – including peripheral blood (mesoderm) and buccal swabs (ectoderm) – to infer soma-wide epigenetic differences is a promising way forward [8,16]. In addition, multiple tissue types can be collected at birth, including cord blood, umbilical cord, placenta, and amnion (birth-associated tissues). The analysis of these heterogeneous tissues shows tissue-specific epigenetic changes, highlighting the importance of considering single-cell-type approaches for a better comprehension of the changes in the target tissue [28–31]. In adult cohorts, the collection of tissues with a higher relevance to disease is limited to biopsy, such as subcutaneous fat or skeletal muscle. Thus, currently available biological materials are effective in relating prenatal exposures with epigenetic differences, and often profit from a large sample size and statistical power for the detection of small differences. Nevertheless, they are less effective in demonstrating that such epigenetic differences actually mediate the association of prenatal exposures with disease outcomes.

Thus, combining conventional approaches with innovative biological material that complement the currently used tissues and address their key limitations can yield significant progress in disentangling the mechanisms underlying the associations between prenatal exposures and later health and disease. Recent studies showed that MSCs have significant potential to increase our insight into the molecular mechanisms that drive the long-term effects of prenatal exposures.

**The favorable characteristics of MSCs**

MSCs are multipotent precursor cells [32] which can be isolated from bone marrow, adipose tissue, and perinatal tissues. In particular, umbilical cord, placenta, amnion, and chorion are well suited for the isolation of MSCs (Figure 1 and Box 1) [32–34]. Their function in vivo remains unclear [35]. Nevertheless, MSCs have shown great potential as therapeutic agents. The potent immunomodulatory properties and differentiation capacity of MSCs is an active field of study in regenerative medicine, which is why there has been much attention on establishing procedures to optimally isolate and culture MSCs from birth-associated tissues (Box 2) [33,36–38]. More recently there has been growing interest in using MSCs to generate insight into the etiology of human disease, specifically in the field of exploring the developmental origins of disease.

Although culturing MSCs is more time-consuming and costly than directly freezing blood and tissue samples (Figure 1 and Box 2), MSCs have three major advantages. First, cultured MSCs are a relatively homogeneous population, and in contrast to traditionally collected birth-associated tissues, they consist of a single cell type. This implies that differences observed in MSCs which are associated with prenatal exposures are unlikely to be produced or obscured by heterogeneity in cell populations. Second, MSCs are self-renewing cells and can be obtained in large numbers. Hence, they can be used for a wide array of analyses from comprehensive epigenomic studies (including histone modifications) to downstream omics (transcriptomics, proteomics), and assays to study functional characteristics of the cells. Third, as multipotent cells, MSCs have the capacity to differentiate into a range of cell types, including adipocytes, chondrocytes, osteoblasts, and neuronal cells (Box 1) [33]. Together, this holds a unique potential for studying the impact of prenatal exposures on the function of cell types that are normally inaccessible in human studies.

MSCs present a promising and versatile toolbox to probe key mechanisms that may contribute to the developmental origins of human disease (Figure 1). We will discuss in greater detail the potential, and also limitations (Box 2), of this toolbox for the study of the omics spectrum, functional
studies, and function of differentiated cells, while reviewing a first wave of literature studying MSCs that illustrates how such studies can break new grounds in the field (Box 2).

**The versatile toolbox MSCs**

**Epigenomics and downstream omics**

With MSCs, the full spectrum of epigenomic changes induced by prenatal conditions can be studied. Instead of relying on DNA methylation analysis alone, other important epigenetic mechanisms can be measured in parallel, as large numbers of viable MSCs can be obtained from a single tissue sampling. Histone modifications and transcription factor binding can be assessed using chromatin immunoprecipitation followed by sequencing, while the 3D chromatin conformation and chromatin accessibility can be measured using assays for chromatin conformation capture techniques such as Hi-C and transposase-accessible chromatin (ATAC) [39,40]. Also, the functional effects of such
gene regulatory changes can be inferred by generating transcriptomics data, for example, using RNA sequencing (RNA-seq) [41]. Finally, it is equally possible to assess the omics levels further downstream—including the proteome, metabolome, or lipidome—to assess the influence of a change in gene regulation on cellular processes using, for example, liquid chromatography–mass spectrometry-based techniques, targeted proteomics approaches, or 2D difference gel electrophoresis [39,42,43]. Correlating prenatal exposures with regulatory and phenotypical cellular differences in MSCs, while following up on the postnatal consequences of the prenatal exposures, can help to gain more insight into the mechanism underlying the developmental origins of disease and potentially highlight affected pathways and processes.

A first series of studies supports the potential of this approach; they reported an association between an adverse intrauterine environment and the epigenetic and transcriptomic profile of MSCs (Table 1). When comparing differences in DNA methylation in human umbilical cord (hUC) MSCs obtained from seven neonates born to obese women versus seven neonates born to non-obese women in a pilot study using the Infinium Human DNA Methylation 450k BeadChip microarray, 5767 CpGs were observed to be differentially methylated [44]. In addition, gene expression and protein levels were assessed in a study identifying differences in PNPLA7, a gene involved in metabolic and developmental processes, at both levels. Whether an adverse epigenetic profile in MSCs can be associated with disease later in life has hardly been investigated thus far. An exception is the methylation state of locus PRKAG2 cg20534694 that could be correlated to neonatal adiposity and cord blood insulin levels [45].

When assessing the transcriptome of hUC-MSCs from six neonates born small for gestational age (SGA), 83 genes were differentially expressed as compared with the control group born appropriate for gestational age [41]. Also, gene-specific qPCR revealed differences in expression upon
experiencing various adverse circumstances during development, such as gestational diabetes mellitus (GDM) or maternal obesity [46–49]. These differences include the expression changes in \textit{RUNX2}, \textit{TP53}, tumor necrosis factor \(\alpha\) (\textit{TNF}), and transcription factor A, mitochondrial (\textit{TFAM}), which are involved in MSC differentiation, senescence, inflammation, metabolism, and mitochondrial functions. High-resolution small RNA-seq of human amnion MSCs (hA-MSCs) derived from children born to obese (\(n=13\)) and non-obese (\(n=7\)) mothers revealed differential expression of 12 miRNAs. miR-138-5p and miR-222-3p were overexpressed most prominently, and are predicted to play a role in the deregulation of cell differentiation, lipid homeostasis, and the response to stress [50].

Whether the difference in gene expression also results in a difference in protein content has been assessed to a lesser extent, but changes in single proteins such as PNPLA7 and RUNX-2 were observed using western blot [44,46]. Additionally, the glycogen synthase kinase (GSK)-\(3\)/\(\beta\)-catenin pathway involved in MSC lineage commitment was found to be altered at a proteomic level in hUC-MSCs derived from children born to obese (\(n=13\)) and non-obese (\(n=7\)) mothers revealed differential expression of 12 miRNAs. miR-138-5p and miR-222-3p were overexpressed most prominently, and are predicted to play a role in the deregulation of cell differentiation, lipid homeostasis, and the response to stress [50].

While various techniques were used to characterize MSCs after adverse prenatal exposures (Table 1), current studies have only scratched the surface of the full potential of MSCs. Histone modifications, DNA accessibility, or the cellular proteomic content are just some of the unexplored options (Figure 2). Comprehensive DNA, RNA, proteome, and metabolome analysis to assess the molecular and functional consequences upon an altered intrauterine environment will be crucial to further elucidate the mechanisms underlying the developmental origins of disease.
MSCs enable the assessment of alterations in cellular function upon exposure to adverse prenatal circumstances and are key to further progress the field. Without assessing cellular function, differences in gene regulation remain merely a marker of change without mechanistic insight. As MSCs are exposed to the milieu in utero, differences in cell characteristics – such as morphology, proliferative capacity, immunophenotype, and cellular metabolism – may reflect the cellular response to the prenatal milieu. Next to this, MSCs allow the assessment of potential functional relationships between genome regulation and cellular functions.

Already, several studies have indicated that the intrauterine environment is reflected in the cellular characteristics of MSCs isolated from perinatal tissues (Table 1). Altered morphology and reduced plastic adherence was observed in hA-MSCs when prenatally exposed to GDM, while hUC-MSCs and human chorion MSCs (hC-MSCs) were reported to retain a normal morphology [49,52]. An increase in the proliferative activity was described for hUC-MSCs derived from neonates diagnosed with SGA [41]. Maternal GDM and obesity appear to have the opposite effect on hUC-MSC proliferation, accompanied by increased cellular senescence [44,49,53,54]. Additionally, a reduction in viability and telomerase activity was indicated in hUC-MSCs upon prenatal exposure to GDM [49,54].

hUC-MSCs and hA-MSCs from neonates born to obese mothers have a distinct immunophenotype, characterized by higher expression of CD13 and CD90, while CD56 expression was lower, as

| Prenatal exposure | Assayed MSC characteristics correlating with prenatal exposure | Observed tissue-specific changes |
|-------------------|-------------------------------------------------------------|---------------------------------|
| Obesity           | DNA methylation                                            | HUC-MSC [Refs]                  |
|                   | Gene expression                                             | HUC-MSC [Refs] |
|                   | Protein abundance                                           | HUC-MSC [Refs] |
|                   | Proliferative capacity/ senescence                          | HUC-MSC [Refs] |
|                   | Immunophenotype                                            | HUC-MSC [Refs] |
|                   | Cell metabolism                                            | HUC-MSC [Refs] |
|                   | Differentiation capacity                                   | HUC-MSC [Refs] |
|                   | Adipogenic: = [44]                                          | Adipogenic: ↑ [48,51] |
|                   | Osteogenic: = [44]                                          | Osteogenic: -- |
|                   | Chondrogenic: = [44]                                        | Chondrogenic: -- |
|                   | Myogenic: = [51]                                            | Myogenic: = |
| Gestational diabetes mellitus | Gene expression                                            | HUC-MSC [Refs] |
|                   | Proliferative capacity/ senescence                          | HUC-MSC [Refs] |
|                   | Telomerase activity/ cell viability                         | HUC-MSC [Refs] |
|                   | Immunophenotype                                            | HUC-MSC [Refs] |
|                   | Cell metabolism                                            | HUC-MSC [Refs] |
|                   | Differentiation capacity                                   | HUC-MSC [Refs] |
|                   | Adipogenic: ↑ [49]                                          | Adipogenic: ↑ [53] |
|                   | Osteogenic: ↓ [49]                                          | Osteogenic: ↓ |
|                   | Chondrogenic: ↓ [49]                                        | Chondrogenic: ↓ |
|                   | Myogenic: = [51]                                            | Myogenic: = |
| Small for gestational age | Gene expression                                            | HUC-MSC [Refs] |
|                   | Proliferative capacity                                     | HUC-MSC [Refs] |
|                   | Cell metabolism                                            | HUC-MSC [Refs] |

The effect of prenatal exposure to obesity, gestational age diabetes, or being born small for gestational age on MSC characteristics is shown. The table includes specification on the tissue of MSC origin in which the changes were observed. This list contains a selection of studies and outcomes and is not a complete list of all performed studies. ↓ and ↑ mark the direction of change, while ↑ marks a general deregulation, and = means no deregulation of the indicated process; – means not reported.
compared with MSCs from neonates born to non-obese mothers \[44,55,56\]. MSCs prenatally exposed to GDM did not show any difference in their immunophenotypes \[49,53\] or migratory and invasive capacity \in vitro\[47\]. A stronger inhibition of mononuclear cell proliferation when cocultured with hUC-MSCs was observed when prenatally exposed to obesity \[44\]. This indicates that experiencing an altered intrauterine environment indeed affects the behavior and phenotype of MSCs.

Metabolic alterations were described in MSCs upon changes in maternal, neonatal, and obstetric factors. A reduction in glycolysis and mitochondrial oxidative phosphorylation, indicated by a lower extracellular acidification rate and oxygen consumption rate, was described for hUC-MSCs derived from neonates of obese mothers \[48,55\]. This was accompanied by a decrease in insulin response, glucose consumption, and lactate production \[48\]. hUC-MSCs isolated from SGA neonates \(n = 3\) showed an increased insulin sensitivity and glucose consumption, compared with a control group born appropriate for gestational age \(n = 3\) \[41\]. Also, GDM-exposed hUC-MSCs display metabolic disturbances such as a decreased oxygen consumption...
rate, lower insulin-mediated glucose uptake, and increased oxidative stress [53,57]. A decreased glucose consumption upon prenatal GDM exposure was also detected in human placental MSCs [58]. This demonstrates that the bioenergetic profile, mitochondrial, and metabolic processes of MSCs are altered and likely resemble the changes in tissues of the fetus that were exposed to the same prenatal conditions.

MSCs can also be used to investigate mechanistic relationships. Several studies have described overexpression and knockdown experiments to link differences in gene expression to alterations in cellular characteristics and processes. Overexpression and knockdown of EGR1, a gene significantly altered in hUC-MSCs of SGA neonates, demonstrated its role in regulating proliferation. EGR1 was found to regulate the expression of COX2, which, when overexpressed, accelerates the glucose uptake in hUC-MSCs of SGA children but not in non-SGA children. Interestingly, the expression pattern of EGR1 could also be correlated to birthweight and fetal abdominal circumference of neonates [41]. Overexpression and knockdown of TP53, a gene differentially regulated in obesity-exposed hUC-MSCs, resulted in differences in cellular senescence, glucose consumption, and lactate production [48]. The silencing and overexpression of the obesity-related gene CD13 showed a functional relation in regulating the adipogenic differentiation capacity of hA-MSCs [56].

Assessing MSCs can therefore link an adverse epigenome to alterations in specific cellular processes (Figure 2). Experimental overexpression and knockdown approaches in conjunction with observational birth cohort studies will be instrumental to pinpoint the precise mechanism underlying prenatally induced disease susceptibility.

Cell differentiation capacity

MSCs can be differentiated into otherwise inaccessible cell types such as adipocytes, chondrocytes, neuronal cells, or cardiomyocytes in vitro (Figure 3). Although best practices for differentiating MSCs derived from birth-associated tissue have yet to be established (Box 2), recent studies show that the differentiation capacity of MSCs depends on prenatal circumstances (Table 1). This offers a unique opportunity to gain tissue-specific insights into the effects of an intrauterine environment.

The adipogenic differentiation capacity was reported to be different in hC-MSCs and hUC-MSC upon prenatal exposure to GDM [49,52,53]. Also, hUC-MSCs and hA-MSCs derived from neonates of obese mothers displayed an increased adipogenic differentiation capacity [48,51,56]. The higher susceptibility for differentiating into the adipogenic lineage was already detectable in undifferentiated MSCs. Here, an increased presence of CD13 was associated with a higher capacity to differentiate towards the adipogenic lineage [51,56]. Moreover, increased phosphorylated β-catenin and decreased inhibitory GSK-3β coinciding with an increased adipogenic differentiation capacity was reported in undifferentiated hUC-MSCs. This finding indicates a regulatory alteration in the GSK-3β/β-catenin pathway, involved in MSC differentiation, upon prenatal exposure to obesity. After adipogenic differentiation, increased lipid staining was associated with the percentage fetal fat and fetal fat-free mass [51]. While promising, this finding could not be reproduced by another study [44], and further studies are warranted to assess whether adipogenic potential is indeed associated with exposure to prenatal obesity.

By contrast, the osteogenic differentiation potential appears consistently decreased in hUC-MSC and hA-MSCs upon prenatal exposure to GDM and obesity [47–49,53]. Additionally, premature birth as well as a low birthweight were linked to a decrease in the osteogenic differentiation capacity of MSCs [46]. The osteogenic differentiation capacity correlated with the RUNX2
expression in undifferentiated MSCs, indicating that differentiation towards the osteogenic lineage may already be detectable prior to differentiation itself.

The chondrogenic differentiation capacity was also reported to be affected by a range of obstetric factors such as managed labor, arterial hypertension, and maternal smoking. These obstetric factors were linked to proteoglycan synthesis, volume of the pellet, and expression of chondrogenic-related genes upon differentiation towards the chondrogenic lineage [59]. hUC-MSCs from GDM pregnancies had a decreased chondrogenic differentiation capacity compared with controls [49,53].

In conclusion, prenatal circumstances have been associated with the differentiation capacity of MSCs in a lineage-specific as well as an origin-specific manner, highlighting the tissue-specific effect of the prenatal environment (Table 1). So far, the sample sizes of the studies using differentiation are moderate, ranging from \( n = 3 \) to \( n = 16 \) per analyzed group. Nevertheless, they illustrate that MSCs provide a promising tool to study the effect of adverse prenatal circumstances on individual tissue development in vitro.

**Epigenomic and functional studies of differentiated MSCs**

Intrauterine exposures can be linked to differences observed in MSCs post differentiation in vitro. Similar to undifferentiated MSCs, the epigenetic spectrum, downstream omics, and cellular functional alterations can be assessed in a variety of differentiated MSCs that may represent
otherwise inaccessible cells types (Figure 3). A small number of studies already made use of this unique potential of MSCs by investigating functional alterations and changes at diverse omics levels. Alterations in cellular metabolism of hUC-MSC-derived adipocytes and myocytes were associated with maternal obesity and neonatal postnatal weight gain [45,60,61].

A study investigating the cellular metabolism of hUC-MSC-derived myocytes upon exposure to maternal obesity reported a disruption in lipid metabolism \((n = 15)\) as compared with controls \((n = 14)\) [45]. This disruption was marked by a higher lipid accumulation and a higher content of the protein peroxisome proliferator-activated receptor \(\gamma\) (PPAR-\(\gamma\)). A decrease in the AMP-activated protein kinase (AMPK) activity was observed, resulting in a reduction in fatty acid oxidation and thereby the accumulation of fatty acids in the hUC-MSC-myocytes. These findings were even more pronounced in UC-MSC-myocytes obtained from neonates showing increased adiposity and cord blood insulin levels. Furthermore, it was indicated that these functional differences were also linked to alterations in the epigenome and transcriptome in MSCs of the same donor prior to differentiation. Already in the undifferentiated state, a hypermethylation in regions known to regulate fatty acid oxidation (such as \(PRKAG2\), \(SDHC\), and \(CPT1A\)) was detected. In line with this, expression of genes associated with the identified differentially methylated regions (DMRs) was distinctly lower. Similar perturbations in lipid metabolism were already identified in adult cells exposed to obesity and animal models [62,63].

A second study compared the energy metabolism in hUC-MSC-derived adipocytes and myocytes from neonates born to obese \((n = 12)\) and lean mothers \((n = 12)\) [60]. A clear correlation between a higher percentage of neonatal fat mass and the increase of several long-chain acylcarnitines and long-chain hydroxyacylcarnitines was identified. In adults, an increase in these metabolites serves as a biomarker for an incomplete \(\beta\)-oxidation and coincides with a compensatory increase in dicarboxylic acylcarnitines. Also, in the hUC-MSC-derived myocytes, the level of dicarboxylic acylcarnitines could be correlated with the percentage of neonatal fat mass and maternal free fatty acid level. However, these findings were restricted to hUC-MSC-myocytes prenatally exposed to maternal obesity and were not found in hUC-MSC-adipocytes derived from the same donor or from donors born to non-obese women. Subsequent high-throughput RNA-seq of hUC-MSC-derived myocytes displaying an incomplete \(\beta\)-oxidation and compensatory \(\omega\)-oxidation revealed an altered gene expression in processes related to metabolism and biosynthesis \((n = 7\), controls \(n = 7\)). Human UC-MSC-adipocytes derived from the same donor show deregulation in genes related to the insulin signaling pathway and other metabolic processes. Maternal BMI and free fatty acid levels were correlated with the downregulation of genes involved in the insulin signaling pathway and AMPK signaling in hUC-MSC-adipocytes.

Along this line, another study \((n = 23)\) identified differences in the lipid and amino acid metabolism in hUC-MSC-adipocytes and -myocytes when comparing children with a rapid gain in fat mass within the first 5 months after birth to neonates with a lower postnatal weight gain [61]. Here, the overabundance of long-chain acylcarnitines found in the hUC-MSC-adipocytes derived from neonates with a rapid postnatal weight gain was even more pronounced in those that were additionally prenatally exposed to obesity. Also, the assessed differences in gene expression were in line with the observed alterations in cell metabolism. Genes involved in the lipid handling, amino acid metabolism, and oxidative stress were differentially regulated comparing hUC-MSC-adipocytes and -myocytes derived from neonates displaying a rapid or low postnatal weight gain.

Hence, differentiated hUC-MSCs exhibit cell type-specific differences when exposed to an adverse intrauterine environment, and these alterations are likely indicative of the development of postnatal conditions such as postnatal weight gain or percentage of neonatal fat mass. This
illustrates that differentiated MSCs can provide a versatile toolbox, allowing investigation of cell type-specific epigenetic alterations and consequential functional changes in response to prenatal circumstances (Figure 3). Follow-up of newborns during childhood and increasing the sample size of studies can shed light on the mechanisms involved in the developmental origins of disease (Figure 1). So far, only a minor fraction of the potential that hUC-MSCs offer has been considered and investigated.

**Concluding remarks**

The intrauterine environment lays the foundation for health throughout life. Prenatal adversity is linked to an increased risk of endocrinological disorders, including obesity, type 2 diabetes, and their cardiometabolic complications. However, the specific molecular pathways mediating this relationship remain largely undefined (see Outstanding questions).

Here, we illustrate that MSCs can be used to set the next step in unraveling the underlying mechanism by combining epigenomics, functional assays, and phenotypic studies. These approaches are frequently impossible in current human studies that are based largely on frozen heterogeneous peripheral tissues such as whole blood. A first wave of reports supports the potential for MSCs as a versatile toolbox to unravel and mechanically link the intrauterine environment to the epigenome, transcriptome, cellular function, and differentiation capacity of MSCs. Their findings highlight the value of using MSCs as a proxy to infer the underlying mechanism of the developmental origins of disease. It will be key to substantially increase the sample size of studies for which MSCs are available to achieve the necessary statistical power and increase the robustness of findings. Since biobanking of MSCs requires more resources than storing whole (cord) blood, it will not be feasible to reach the sample sizes currently analyzed with whole (cord) blood samples in the near future. However, if birth cohorts with ongoing sample collection start collecting MSCs to subsets in addition to cord blood, sufficiently large sample sizes can be achieved in consortia. It will be collaborative efforts that pave the way for the next generation of personalized preventative strategies that focus on the beginning of life but last a lifetime.

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No interests are declared.

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**Outstanding questions**

What mechanisms mediate the association between the intrauterine environment and the risk of obesity, type 2 diabetes, and other metabolic diseases later in life?

Which epigenomic marks beyond DNA methylation, downstream omics level, and cellular processes are altered upon exposure to an adverse prenatal environment?

Is the effect of prenatal exposures tissue-specific and, if so, what surrogate model may be used to study such effects while the tissues of actual interest are inaccessible?

How can we further optimize protocols for the collection, characterization, and differentiation of MSCs to support larger-scale studies in birth cohorts to answer these questions?

What biomarkers can detect exposure to prenatal adversity early in life and predict an increased susceptibility of metabolic diseases later in life?
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