Developmental Programming Through Epigenetic Changes

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The last decade has seen an exponential number of scientific articles addressing the concept of fetal programming of adult onset diseases. Though it has long been known (as it is probably part of common knowledge in human history) that the environment and life events during the fetal period impact on the child’s development, the notion that intrauterine life can also impact on adult health and on the incidence of late onset diseases such as hypertension and type II diabetes is relatively more recent. In 1964, Rose reported the association of ischemic heart disease and infant mortality within the same families (reviewed by1). Then Forsdahl in 1977 showed that incidence of arteriosclerotic heart disease in a certain age group could be correlated with the infant mortality rate of that same population.1 Interest in these types of association rose dramatically following Professor Barker and collaborators’ extensive observations of a well documented population in England and Wales. The meticulous work of home health visitors in the early 20th century, which noted every child weight and length at birth and throughout the first year of life as well as other observations of the mother health and living milieu, generated data which clearly demonstrated an inverse relationship between birthweight and adult incidence of cardiovascular related mortality and morbidities as well as type II diabetes.1

We will focus here on hypertension and related complications. The association between birthweight and adult blood pressure was demonstrated in many parts of the world, in industrialized as well as in more developing countries, in both men and women.1 The association is first noted in early childhood (but not at birth) and seems to increase with age. Despite the significant number of studies published, which have examined to date tens of thousands of subjects, the mechanisms linking fetal life and adult diseases are contrastingly incompletely understood.

Poor nutrition was initially the main suspected causal factor precipitating the events leading to adult hypertension because the highest incidence of cardiovascular related diseases was found in the lowest birth weight group which also represented the lowest socioeconomic environment and living conditions. These observations led to the hypothesis that poor nutrition in fetal life alters permanently the developing organs leading to diseases in adult life.1 To test this hypothesis, animal models were developed using global caloric or specific nutrient restriction of the pregnant animal (see extensive review2), such as the low protein diet studied by the investigators Bogdarina et al in the current issue of Circulation Research3. The main mechanistic hypothesis has long been that when a fetus is developing under suboptimal conditions, it adapts to guarantee immediate survival and to preserve vital functions at the expense of others less immediately critical functions. For example, inducing peripheral insulin resistance to preserve glucose for vital organs while halting the development of others such as the kidneys or the pancreas. However the fetus metabolic “economy” mode that ensures survival in response to prenatal adverse conditions becomes maladaptive in a postnatal world, which is characterized by an abundance of nutritional resources. Supporting this theory is the observation that the cardiovascular outcome is notably worse in subjects who are one born short and thin and grow to become short and chubby, than in individuals who remain short but thin into adulthood. This underlines the importance of postnatal life in its role of further modulating antenatal programming.4

Mechanistic pathways that have been extensively studied in this context include glucocorticoid exposure, role of the kidneys and of the renin angiotensin system. Excess exposure of the fetus to maternal glucocorticoids is a key player.5 The enzyme 11β-hydroxysteroid dehydrogenase type II (11β-HSD II) is abundant in the placenta and metabolizes cortisol into inactive cortisone. The expression of 11β-HSDII is decreased in human placenta exhibiting intrauterine growth retardation, as well as in dams fed a low protein diet, resulting in increased exposure of the fetus to active maternal glucocorticoids.5 Supporting this, elevation of blood pressure in the low protein rat model is prevented by maternal blockade of glucocorticoids synthesis.5

The role of the kidneys and of the renin angiotensin system into fetal programming of hypertension is an active field of investigation. A decrease in nephron numbers is observed in autopsies of hypertensive humans,6 and in rat offspring of dams fed a low protein diet.7 This structural impairment is associated with abnormal Na handling by the kidneys.8 As cited by the authors, hypertension in low protein offspring is prevented by blockade of angiotensin II formation from 2 to 4 weeks of life; this long-term effect on blood pressure is not observed when adult offspring of low-protein pregnancy are treated.7 Elevated blood pressure is also normalized by central blockade of angiotensin II effects in low-protein offspring along with a significant increase in the expression of AT1 receptor expression in brain cardiovascular regulating areas.9 Taken together, these data demonstrate a major tonic
role of peripheral and brain renin angiotensin system in maintaining hypertension that was programmed in utero. Factors leading to enhanced renin angiotensin system activity are unknown but corticosteroids are a good candidate. Blood pressure responsiveness to angiotensin II but not to noradrenaline is enhanced in fetal sheep after cortisol infusion. In adult rats, glucocorticoids increase AT1, mRNA expression in specific brain regions implicated in central cardiovascular control as well as in vessels.

Programmed hypertension associated with exposure to a low protein diet is associated with vascular dysfunction. In addition to impaired NO-mediated vasorelaxation, vascular dysfunction in adult animals is characterized by enhanced vasomotor response to Ang II both in vivo and in isolated peripheral vessels, secondary to increased vascular superoxide production by NADPH oxidase with concomitant increase of Ang II AT1 receptor subtype. Which AT1 receptor subtype is upregulated however has not been examined. Reports showing that AT1b might play a role in vasoconstriction at the pre junctional level (postganglionic sympathetic neurons innervating vascular smooth muscle cells) could support the hypothesis presented by Bogdarina et al. of a role for AT1b in programmed hypertension; however vascular expression of AT1a versus AT1b was not examined in this article.

AT1 is responsible for most of the known functions of the renin–angiotensin system in both physiological and pathophysiological conditions: vascular smooth muscle contraction, proliferation of vascular smooth muscle cell, aldosterone release and regulation of fluid electrolyte balance. Two subtypes of AT1 receptor have been identified in rodents: AT1a and AT1b. These receptors are more than 95% identical at the amino acid level and pharmacologically indistinguishable. However there is low homology (35%) in the 5'- and 3'-untranslated regions of the AT1a and AT1b mRNAs; therefore the distribution of the receptors subtypes in tissues has been studied using mostly RT-PCR and in situ hybridization. In the adult rat, AT1a receptor predominates in the liver, lung, aorta and at all nephron segments except the glomeluri. AT1a is expressed in many areas of the brain including those involved in blood pressure and fluid homeostasis whereas AT1b is virtually absent from all the brain structures. In the zona glomerulosa of the adrenal gland and the mesangial cells of the nephron both AT1a and AT1b are present. Mice with a targeted disruption of the AT1b receptor show no modification of their organ histology including adrenal zona glomerulosa and kidneys, have blood pressure similar to their wild-type litter mates as well as comparable plasma aldosterone levels. Moreover these mice do not display compensatory enhancement of AT1a in their kidneys and adrenals. Mice null mutated for the AT1b receptor have significantly decreased blood pressure, do not increase their blood pressure after renal artery clipping, and AT1 specific binding in the kidneys is nearly undetectable. However these mice can mount a blood pressure response to angiotensin II infusion, even though it is an order of magnitude less than the response in wild-types, suggesting that AT1b can cause a significant pressor response. AT1a−/− mice can also significantly increase their vascular superoxide production in response to angiotensin II. Surprisingly renal structure at AT1a null mutated mice is normal at birth and they do not develop severe renal microvascular disease and tubulointerstitial injury observed in the angiotensinogen- and angiotensin converting enzyme-deficient mice, as well as in the double knock out AT1a and AT1b mice. These studies indicate that AT1b does not play a predominant role in blood pressure maintenance and other functions attributed to activation of AT1 receptor by angiotensin II in normal conditions, but could play a compensatory role in specific situations. It is interesting to note however that AT1b seems to mediate vasoconstriction to angiotensin II in conductance (but not resistance) vessels of the mouse. The authors here demonstrate changes in expression and DNA methylation marking of the AT1b gene, which seem to be less important than AT1a in normal stressor response. Although there is evidence that AT1b might have a pressor function, further evidence is required before we can conclude that epigenetic changes in programming of AT1b gene expression in the adrenal play a role in the development of programmed hypertension.

Oxidative stress has more recently been incriminated in long-term programming in response to low protein pregnancy. The hypothesis that oxidative stress may be the initiating trigger for developmental programming of hypertension is supported by the following experimental evidence. The spontaneously hypertensive rat (SHR) is considered a genetic model of hypertension in which however a role for neonatal environmental factors in hypertension development is suggested; supplementation of SHR during gestation and early post natal weeks with antioxidants resulted in persistent reduction of adult blood pressure. In a recent study, we reported that administration of the peroxidation inhibitor lazaroid to low protein fed dams prevents elevation of blood pressure and vascular dysfunction in the offspring.

What is the mechanism by which prenatal exposure to adverse conditions results in long-term effects during adulthood? It is now clear that prenatal exposures result in stable life-long changes in expression of genes such as the increase in AT1b expression in the kidney reported here, but how are these changes in programming of gene expression generated by the adverse prenatal environment and how are they maintained through life long after the adverse exposures are gone? The manuscript here examines the hypothesis that intrauterine adversity resulted in altered epigenetic markings of the AT1b gene by DNA methylation. The expression of genes is defined by their epigenetic state, which includes chromatin structure and the state of modification of histones as well as modification of DNA by DNA methylation. Methylation in critical regulatory regions is believed to mark silenced genes. DNA methylation is a stable and long term signal. Thus, it is proposed here that intrauterine adversity resulted in partial loss of methylation from critical SP1 sites in the promoter of the AT1b gene. This pattern of methylation is maintained into adulthood resulting in hypomethylation and elevated expression of the AT1b gene through life. Thus, DNA methylation might be a mechanism, which stably fixes the transient exposure to adverse conditions in early life to a stable change in gene expression programming long after the first exposure is gone. A similar model has been recently
proposed from the programming of the expression of the glucocorticoid receptor in response to maternal care during early life.22

Methylation was traditionally believed to be a fixed on-off signal that serves to completely silence genes in the appropriate tissue.23 The pattern of methylation observed in the zona glomerulosa of control animals is partial. Not all the sequenced genes are methylated. This partial methylation might result from cell-specific variations in the state of methylation and expression of AT1b even in this relatively defined and uniform population of cells. Thus, even in control animals some cells have methylated AT1b genes and do not express the gene whereas other cells have unmethylated genes and express the AT1b mRNA. If this is true, then the adrenal defines its normal AT1b response by determining the fraction of cells which express this receptor through methylation of the gene. In prenatally protein-restricted animals this fraction is increased as a result of an increase in the fraction of the unmethylated genes. Another possibility is that the methylation pattern is dynamic in all cells and that the observed pattern represents the dynamic steady state of methylation in the population and that this steady state is altered in response to nutritional restriction during pregnancy. Thus, in any given cell the gene could be either methylated or unmethylated at any given point in time. The probability of DNA methylation is altered by prenatal protein restriction

The results described here provide an example of the utilization of DNA methylation as a mechanism to fine-tune physiological responses. It was long believed that DNA methylation plays a role in cellular differentiation and determination of cell identity,23 this data however is a nice illustration of the possibility that DNA methylation plays a physiological role as well. Many questions remain to be addressed in future experiments. The mechanisms leading from nutritional restriction to specific changes in DNA methylation are unclear, nor is the functional role of this methylation pattern is dynamic in all cells and that the probability of DNA methylation is altered by prenatal protein restriction. In addition, there is the question of the transgenerational transmission of this phenotype. One possibility is that the programmed mother which also has vascular dysfunction will provide a deprived intrauterine environment to its offspring, thus perpetuating the cycle of fetal (ma)adaptation. An alternative possibility is that epigenetic modification of the germ line by stable methylation caused by the prenatal exposure will transmit the prenatal experience of one generation to future generations. This idea raises the provoking possibility of nongenetic heritable changes in phenotype.

Although epigenetic modifications were proposed as probable mechanisms of cardiovascular and metabolic programming, there are relatively few data to date that demonstrate epigenetic changes. The biologists nevertheless have since long realized the importance of the environmental conditions in the phenotypic determination of a species offspring. Striking examples can be found in the insect and plant worlds such as the colors of a butterfly wings which are modified in accordance with the season of the larval period.24 Epigenetic changes and programming: many candidates and confirmed players able to induce developmental programming of hypertension are also known to modify gene methylation: Rees et al.25 showed hypermethylation in the liver of fetuses from low protein fed dams. PPAR α and glucocorticoid receptor genes are hypomethylated and their expression increased in the liver of young offspring of low protein fed dams.26 Reactive oxygen species can modify methylation leading to changes in gene transcription and protein expression.27 Work by Meany and Szyf have shown epigenetic changes leading to changes in the expression of the glucocorticoid receptor depending on mother behavior toward its pups.28–30

One issue of prime importance is that epigenetic changes in difference from genetic changes are potentially reversible. Thus they introduce the concept of change to the deterministic world of genetics and offer the hope of intervention to remove deleterious epigenetic marks. There is some evidence that epigenetic programming by early life events is potentially reversible by certain pharmacological manipulations later in adulthood.29–31 This challenges the notion that these changes are induced during fetal life and immutable thereafter; it is critical to take this possibility into account when interpreting epigenetic changes observed in animal models of developmental programming of hypertension or when making association in humans between perinatal factors and epigenetic changes of key genes.

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References


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