The fetal origin of adult atherosclerosis : a study in ApoE and Ldlr mouse models

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In this thesis, the long-term consequences of an adverse intrauterine environment, created by maternal apoE-deficiency or Ldlr-deficiency, on adult athero-susceptibility in the offspring has been explored. In addition, the epigenetic mechanisms underlying the acquired susceptibility for cardiovascular disease were investigated.

Chapter 1 provides a general introduction on cardiovascular disease and the major environmental risk factors involved in atherosclerosis. Furthermore, the possible fetal origin of cardiovascular disease and the role of epigenetic programming in this process are described.

In **Chapter 2**, the effect of maternal apoE-deficiency, characterized by hypercholesterolemia, high oxidative stress and inflammation, on fetal vascular development and adult cardiovascular disease risk in apoE$^{+/-}$ offspring has been investigated. In late fetal stages, a slight, but significant plasma cholesterol elevation was found in apoE$^{+/-}$ fetuses from apoE$^{-/-}$ mothers in comparison to apoE$^{+/-}$ fetuses from wild-type mothers. In addition, morphological and morphometric analysis revealed profound vascular injury, characterized by a significant increase in loss of endothelial cell volume, in the carotid arteries of fetuses from apoE$^{-/-}$ mothers. After birth, the damage to the endothelial cell lining was repaired and no pathology could be detected. Constrictive collar placement around the left common carotid artery of adult apoE$^{+/-}$ offspring from apoE$^{-/-}$ mothers which were fed a cholesterol-containing diet, resulted in severe neointima formation in 9 out of 10 mice analyzed. In apoE$^{+/-}$ offspring from wild-type mothers, we only detected minor lesion volume (2 out of 10). Since neointima formation in apoE$^{+/-}$ offspring from apoE$^{-/-}$ mothers was accelerated and aggravated in relation to genetically identical offspring from wild-type mothers, it can be concluded that maternal apoE-deficiency during pregnancy has adverse effects on cardiovascular disease risk in the adult offspring.

Chapter 3 also concerned the apoE$^{+/-}$ mouse model. Here we investigated whether adult apoE$^{+/-}$ offspring from apoE$^{-/-}$ mothers was also more susceptible to neointima formation in absence of a cholesterol-containing diet. On a chow diet, apoE$^{+/-}$ offspring from apoE$^{-/-}$ mothers, as well as apoE$^{+/-}$ offspring from wild-type mothers were normocholesterolemic. The results showed that intrauterine exposure to maternal apoE-deficiency was sufficient to allow collar-induced neointima formation, even without cholesterol feeding. The neointimal lesions detected in

apoE$^{+/-}$ offspring from apoE$^{-/-}$ mothers were small, but present, sustaining the hypothesis that an adverse intrauterine environment provided by maternal apoE-deficiency poses a major risk factor for adult disease.

In **Chapter 4** the role of maternal hypercholesterolemia in intrauterine programming of adult cardiovascular disease risk was studied in the Ldlr$^{-/-}$ mouse model. In Ldlr$^{-/-}$ offspring, intrauterine exposure to maternal hypercholesterolemia resulted in fetal intimal thickening and profound spontaneous atherosclerosis in the adult stage. Maternal hypercholesterolemia appears to have limited effects on athero-susceptibility in Ldlr$^{+/-}$ offspring. Analysis of the fetal vasculature and cholesterol levels revealed no adverse effects when compared with Ldlr$^{+/-}$ fetuses from Ldlr$^{+/-}$ mothers. In addition, collar placement in adult Ldlr$^{+/-}$ offspring from Ldlr$^{-/-}$ mothers did not accelerate and aggravate neointima formation in relation to Ldlr$^{+/-}$ offspring from Ldlr$^{+/-}$ mothers. Here, no lesions could be induced at all. Solely in spontaneous atherosclerosis in Ldlr$^{+/-}$ mice from Ldlr$^{-/-}$ mothers, a difference in aortic medial hypertrophy was detected suggesting that maternal hypercholesterolemia is not the primary trigger for intrauterine programming. In conclusion, we can state that most likely maternal hypercholesterolemia has a more regulatory role in this process.

In **Chapter 5** the capacity of adult endothelial cells to undergo endothelial-to-mesenchymal transformation in vivo was explored. Morphological analysis of femoral arteries of Tie2LacZ mice 3 days after nonconstrictive collar placement revealed endothelial detachment and proliferation. As early as 7 days after collar placement, β-galactosidase, which is endothelial cell-specific, could also be detected in the neontima. A number of these β-galactosidase-positive mesenchymal cells gained positivity for α-smooth muscle actin. The β-galactosidase-positive, α-smooth muscle actin-positive mesenchymal cells most likely are of endothelial cell origin. The contribution of these cells to the developing neointima suggests a role for endothelial cell derivatives in the development and progression of cardiovascular disease.

Chapter 6 is a review that focuses on the maternal signals that may cross the placental barrier and exert detrimental effects on embryonic and fetal development with a subsequent cardiovascular disease risk. Fetal undernutrition, maternal hypercholesterolemia and a number of other maternal factors all have been associated with an increased risk for cardiovascular events. Alterations in normal programming during sensitive embryonic or fetal developmental periods may result in the establishment of increased atherosclerosis susceptibility that persists into

adult life. Growing evidence from animal studies indicates that the underlying epigenetic mechanisms comprise DNA methylation or histone modifications.

Chapter 7 shows that profiles of histone triple-methylation modifications of lysines and the accompanying lysine methyltransferases in endothelial cells and smooth muscle cells were clearly differentially affected in apoE$^{+/-}$ offspring from apoE$^{-/-}$ mothers in relation to apoE$^{+/-}$ offspring from wild-type mothers. Thus, it seems that intrauterine programming of susceptibility for cardiovascular disease in adult life is (in part) established by adverse influences of maternal apoE-deficiency on chromatin remodeling in the vasculature of the offspring.

Chapter 8 provides a general discussion of the data presented in this thesis. The role of maternal hypercholesterolemia, oxidative stress, and alterations in the immune system in prenatal programming of adult cardiovascular disease is discussed. In addition, the epigenetic mechanisms through which these risk factors may exert their effects are described. Finally, where possible, a link with the human situation is made and the implications of the data presented in this thesis on prevention and treatment strategies in pregnant women and offspring are presented.

In summary, in this thesis it becomes clear that the intrauterine environment created by the mother during pregnancy not only has beneficial effects on the developing embryo / fetus. Although it is too early to draw definite conclusions, the first results of this research line show that maternal apoE-deficiency, in contrast to maternal Ldlr-deficiency, adversely affects the offspring, not only in late fetal stages but also in adult life. Our data indicate that the inflammatory status of the mother and the lack of maternal apoE itself may attribute to the increased cardiovascular disease risk observed in the adult offspring. Hypercholesterolemia and oxidative stress possibly play a more regulatory role. In a first attempt to elucidate the underlying mechanism we show that maternal apoE-deficiency leads to changes in the histone triple-methylation modifications in the vascular wall of the offspring. This can be considered an important lead that needs to be investigated further. It does not mean, however, that we are close to complete elucidation of the underlying mechanism. A lot of research is needed to accomplish this and it is needed. Why? The fact that a hit so early in life exerts negative effects on cardiovascular disease risk in adulthood is worrisome. If we could succeed in elucidating the exact role of epigenetics in this process and are able to translate these data to the human situation, possibly we could reduce the incidence of cardiovascular disease.