Short Communication

Renal Failure Increases Cardiac Histone H3 Acetylation, Dimethylation, and Phosphorylation and the Induction of Cardiomyopathy-Related Genes in Type 2 Diabetes

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The combination of diabetes and renal failure is associated with accelerated cardiomyopathy, but the molecular mechanisms of how renal failure drives diabetic heart disease remain elusive. We speculated that the metabolic abnormalities of renal failure will affect the epigenetic control of cardiac gene transcription and sought to determine the histone H3 modification pattern in hearts of type 2 diabetic mice with several degrees of renal dysfunction. We studied the histone H3 modifications and gene expression in the heart of 6-month-old nondiabetic mice and type 2 diabetic db/db mice that underwent either sham surgery or uninephrectomy at 6 weeks of age, which accelerates glomerulosclerosis in db/db mice via glomerular hyperfiltration. Western blotting of hearts from uninephrectomized db/db mice with glomerulosclerosis, albuminuria, and reduced glomerular filtration rate revealed increased acetylation (K23 and 9), phosphorylation (Ser 10), dimethylation (K4), and reduced dimethylation of (K9) of cardiac histone H3 as compared with db/db mice with normal renal function or nondiabetic wild-type mice. This pattern suggests alterations in chromatin structure that favor gene transcription. In fact, hearts from uninephrectomized db/db mice revealed increased mRNA expression of multiple cardiomyopathy-related genes together with cardiomyocyte hypertrophy. These data suggest that renal failure alters cardiac histone H3 epigenetics, which foster cardiomyocyte hypertrophy in type 2 diabetes. (Am J Pathol 2010, 176:1079 –1083; DOI: 10.2353/ajpath.2010.090528)

Early diabetic nephropathy, affecting more than 30% of diabetes patients, is characterized by glomerular hyperfiltration and increased production of extracellular matrix but tends to progress to diffuse glomerulosclerosis, proteinuria, and renal failure.1 Proteinuria and renal failure are two independent risk factors for cardiovascular complications in type I and type II diabetes.2 Diabetic cardiomyopathy is characterized by cardiomyocyte hypertrophy, perivascular or interstitial fibrosis, and interstitial accumulation of glycoprotein.3 The molecular pathways that link renal failure to progression of cardiac disease remain unclear. Several studies have proposed a role of hypertension,4 dyslipidemia,5 activation of the renin-angiotensin system,6 endothelial dysfunction,7 oxidative stress,8 and inflammation9 in this context. It is thought that all of these factors affect the function and finally the structure of the cardiac vasculature and cardiomyocytes by activating different signaling pathways that specifically drive transcription of downstream pathogenic factors.3

Histone epigenetics are now recognized as another level of gene transcription control because covalent histone modifications regulate chromatin dynamics like heterochromatin formation as a requirement for transcription factor binding.10,11 For example, histone deacetylase 5 reduces histone H3 acetylation and thereby impairs the expression of cardiomyocyte growth and remodeling...
Table 1. Primer Sequences Used for Real-Time RT-PCR

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<th>Gene name</th>
<th>Accession No.</th>
<th>MGI nomenclature</th>
<th>Primer sequences</th>
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MGI: Mouse Genome Informatics.
time RT-PCR was performed on a Light Cycler 480 (Roche) using Sybr Green PCR master mix and the primers as listed in Table 1. Gene expression values were normalized for respective 18s RNA expression.

Histone Extraction and Immunoblotting

Hearts were manually dissected and histone isolation was performed as described. Three hearts were pooled from each group for histone isolation. Immunoblot analysis was performed by using anti-acetylated histone H3 at lysine 23 and 9 (rabbit 1:5000), anti-phosphorylated histone H3 at serine 10 (rabbit 1:5000), dimethylated histone H3 at lysine 4 and 9 (rabbit 1:5000), anti-histone H3 (rabbit 1:5000), and horseradish peroxidase-conjugated anti-rabbit secondary antibodies (all from Cell Signaling Technology, Danvers, MA). Proteins were detected with the enhanced chemiluminescence system and enhanced chemiluminescence Hyperfilm (Amersham, Freiburg, Germany). Immunoblots were quantitated by densitometric analysis and the exposures were in linear dynamic range. The densitometry analysis was performed by Image J software, NIH, USA.

Statistical Analysis

Data are presented as mean ± SEM. Comparison of groups was performed using Student t test and one-way analysis of variance followed by Dunnett test. A value of $P < 0.05$ was considered to indicate statistical significance.

Results

Early Uninephrectomy Increases Albuminuria and Reduces GFR in db/db Mice

Early uninephrectomy induces glomerular hyperfiltration, which enhances the progression of diabetic glomerulosclerosis in male db/db mice. As such early uninephrectomy increased the glomerulosclerosis score and albuminuria and reduced GFR in 6-month-old db/db mice (Table 2). By contrast, uninephrectomy did not affect body weight or plasma glucose levels (Table 2). Hence, uninephrectomized male db/db mice represent a model of type 2 diabetes with diffuse glomerulosclerosis, albuminuria, and renal failure.

Renal Failure Affects Epigenetic Histone H3 Modifications in Hearts of db/db Mice

We used Western blotting of cardiac cell nuclei extracts to determine a number of specific covalent histone modifications. We first studied the impact of diabetes by comparing hearts of sham-operated C57BL/6 with those of sham-operated C57BLKS db/db mice at the age of 24 weeks. The latter showed increased H3 acetylation at lysine 9 and 23, H3 dimethylation at lysine 4 and 9, and H3 phosphorylation at serine 10 (Figure 1). Early uninephrectomy further increased H3 acetylation at lysine 9.
and 23, H3 dimethylation at lysine 4, and H3 phosphorylation at serine 10 as compared with 2K db/db mice (Figure 1). We also observed a decrease in the H3 dimethylation at lysine 9 in 1K vs 2K db/db mice or wild-type mice (Figure 1). By contrast, none of these changes was observed in cardiac histone preparations from 1K wild-type mice (Figure 2). Hence, kidney disease in type 2 diabetic mice alters histone H3 epigenetics in a way that indicates transcriptionally active chromatin.

Renal Failure Enhances Cardiac mRNA Expression of Several Cardiomyopathy-Related Genes and Induces Cardiomyocyte Hypertrophy in db/db Mice

Activation of cardiac chromatin may be associated with increased expression of cardiomyopathy-related genes. We found that the mRNA levels of myosin heavy chains 3, 6, and 7, myosin light chain 3, as well as tubulin-α, catenin-α, and laminin-β2 were significantly increased in hearts of 1K versus 2K db/db mice (Figure 3A). This was associated with the induction of a number of additional genes known to be involved in tissue remodeling (e.g., matrix metalloproteinase 1, plasminogen protein, Riken, and vascular endothelial growth factor β). By contrast, diabetes in 2K db/db mice did only affect MHC myosin heavy chain mRNA levels as compared with non-diabetic wild-type mice (Figure 3A). Next, we questioned whether the altered histone H3 modification pattern and the increased gene expression in hearts of 1K db/db mice would be associated with a distinct cardiomyocyte phenotype. To answer this question, we performed histomorphometrical analysis of crosssections of the anterior left ventricular wall and the interventricular septum from mice of all groups. In 6-month-old 1K db/db mice
the number of cardiomyocyte nuclei in a given high-
power field was significantly reduced as compared with
age-matched 2K db/db mice, which was already signifi-
cantly lower as compared with that of nondiabetic wild-
type mice (Figure 3B). These data would support that
renal failure increases cardiomyopathy-related gene ex-
pression and cardiomyocyte hypertrophy rather than car-
diomyocyte proliferation. Heart weights and heart to body
weight ratios did not significantly differ between 2K and 1K
db/db mice (Table 2).

Discussion
We found renal failure to be associated with increased
cardiac histone H3K9 and H3K23 acetylation, H3K4 dim-
ethylation, and phosphorylation at serine 10. The effect
of renal failure adds to that of type II diabetes as the com-
parison of diabetic and nondiabetic mice already re-
vealed a similar modification pattern. However, unine-
phrectomy did not affect cardiac histone epigenetics in
wild-type mice, which may be a relevant finding in the
context of living kidney donation. It is of interest that
histone acetylation, H3K4 dimethylation, and phosphory-
lation at serine 10 lead to histone relaxation (ie, unwind-
ing of the packed nucleosomes), which makes the DNA
accessible for the binding of activated transcription fac-
tors that are translocated into the nucleus. 10,11,17,18 By
contrast, H3 dimethylation at lysine 9 promotes chromatin
condensation, which suppresses transcription factor
binding. 11 H3 dimethylation at lysine 9 has recently been
reported to be suppressed in bovine aortic endothelial
cells after transient glucose exposure. 19 We did not ob-
serve the same in hearts of db/db mice with persistent
hyperglycemia but H3 dimethylation at lysine 9 was sup-
pressed in 1K db/db mice. Together, the epigenetic hist-
one H3 modification pattern observed in hearts of db/db
mice with renal failure would predict increase in global
gene expression (eg, in cardiomyocytes). We therefore
analyzed the mRNA expression of cardiomyopathy-re-
lated genes because cardiomyocyte hypertrophy has
been reported as one of the features of diabetic cardio-
myopathy. 3 Our finding that renal failure increased car-
di myocyte mRNA expression of most of these genes together
with cardiomyocyte size in db/db mice might be a direct
result of this specific epigenetic histone modification pat-
tern. Yet it remains unclear which is the most relevant
factor modulating histone epigenetics, and future work
will need to determine how factors like arterial hyperten-
sion, hyperglycemia, hyperinsulinemia, obesity, protein-
uria, and uremic toxins individually affect histone modi-
fying enzymes. In addition, it will be necessary to see
whether histone epigenetic-modulating drugs can pre-
vent cardio-renal syndromes, like diabetic nephropathy-
associated cardiomyopathy. 20,21 Furthermore, it is likely
that the observed changes do also occur in other organs
affected by diabetes complications, a topic to be studied
in the future.

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kinase p38 expression by curcumin in streptozotocin-induced type I
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