

INVITED PAPER

THE ROLE OF EPIGENETICS IN KIDNEY DISEASES

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Abstract: Epigenetics is the study of changes in gene expression that occur without changes in DNA sequence. These reversible modifications include DNA methylation, histone acetylation and RNA interference. Epigenetic changes are fundamental for the regulatory physiological processes that regulate gene activity; therefore, these changes are altered in response to environmental factors or disease states, but, in many circumstances, they are also believed to contribute to disease occurrence and progression. So far, studies of the epigenome have been scarce in nephrology. However, there is evidence that in the uremic milieu, several features such as inflammation, dyslipidaemia, hyperhomocysteinaemia, oxidative stress as well as vitamin and nutritional deficiencies may affect the epigenome, and impact patient outcome. The present review describes the current knowledge on epigenetic alterations in the course of various kidney disease states. Although the science of epigenetics is still in its infancy, it seems that it may help elucidate the pathogenic mechanisms in uraemia, and allow novel treatment strategies to be developed.

Key words: epigenetics, kidney disease, DNA sequence, DNA methylation, histone acetylation, RNA interference.

Introduction

The term ‘epigenetics’ refers to mechanisms that initiate and maintain changes of gene expression and gene function without changing the sequence of

the genome [1, 2]. In eukaryotes, DNA is packaged into chromatin, a complex which, apart from DNA, consists of histone and non-histone proteins. The accessibility of chromatin is essential for gene expression, and is considerably regulated by the epigenetic status. Epigenetic regulation of gene expression involves postreplicative DNA methylation, RNA interference, and posttranslational histone modifications. Epigenetic modulation of gene expression often results from environment – gene interactions. It leads to heritable (through cell division), but potentially reversible, changes in production of the final protein product of the gene. Studies of various species have shown that epigenetic control is critical for the normal function of the genome and that its alterations may promote disease [3].

Among epigenetic modifications, DNA methylation has attracted most interest due to the relative ease by which it can be studied, and as it regulates fundamental biological phenomena such as genome stability and gene expression [4]. DNA methylation involves a postreplicative covalent DNA modification, whereby a methyl group is added to cytosine residues in a CpG dinucleotide. In general, DNA methylation suppresses gene expression by modulating the access of transcription factors to the chromatin [5]. Indeed, it has been documented that transcriptionally active regions of DNA are highly unmethylated, whereas the repressed regions are comprised of methylated DNA [3]. It is now well established that major alterations in DNA methylation patterns, and associated gene activities, are often present in various disease states, especially in cancer [6].

Other mechanisms that are mostly referred to as epigenetic, include histone modifications resulting in chromatin remodeling, and RNA interference. Addition of acetyl groups to the N-terminal tail of a histone octamer reduces the affinity of histones for DNA and allows RNA polymerase and transcription factors to access the promoter [7]. In contrast, histone deacetylases are responsible for removal of acetyl groups, which generally results in transcriptional repression. The processes of DNA methylation and histone acetylation are often associated, controlling the activity of transcription and, hence, gene expression [7]. RNA interference consists of gene expression inhibition through degradation or translational inhibition of mRNA by microRNAs (miRNAs), a large family of small, approximately 21-nucleotide-long, non-coding RNAs [8].

Analysis of the epigenome

Since epigenetic modifications and their influence on gene expression are tissue-specific, assessment of the epigenome requires access to the tissue/cell type of interest. Obviously, this makes many epigenetic investigations in human studies difficult, if not impossible. In some cases, surrogate tissues and/or

autopsy material have to be used. The assessment of DNA methylation can be performed on most biological materials. It can be evaluated at the genome-wide global scale, or gene-specific. Several methods for DNA methylation analysis have been used [9]. Global DNA methylation can be assessed in large patient materials using the luminometric assay (LUMA) [10]. Global analysis has to be regarded as a blunt tool, but it should be emphasized that aberrant global DNA methylation is a sign of an epigenetic deregulation that may provide important information of general disease states.

Epigenetics and kidney diseases

Emerging evidence suggests that epigenetic alterations are involved in the pathogenesis of diseases predisposing to chronic kidney disease (CKD). DNA hypermethylation has been associated with predisposition to, and progression of, atherosclerosis [11]. Moreover, experimental studies have demonstrated the importance of histone modifications in atherosclerosis development [12, 13]. A recent study that compared site-specific DNA methylation levels in more than 14,000 genes between Hispanic diabetes and African-American end stage renal disease patients showed that DNA methylation differences were associated with disease predisposition and/or treatment [14]. Based on their findings, the authors speculate that inter-individual epigenetic differences may prove useful as predictive biomarkers of disease susceptibility. Epigenetic silencing of genes encoding for transcription factors regulating insulin gene expression and beta cell differentiation have been implicated in the onset of type-2 *diabetes mellitus* [15]. It is also acknowledged that epigenetic modulations of gene expression by changes in DNA methylation and histone acetylation might predispose to hypertension [16]. Among numerous other examples, it is believed that epigenetic alterations contribute to the pathogenesis of systemic *lupus erythematosus* [17] and are associated with ANCA vasculitis [18].

Since various environmental factors may modify the epigenetic state, it would not be surprising if the toxic uraemic milieu *per se* would have an impact on the epigenome and the regulation of systemic homeostasis. Indeed, hyperhomocysteinaemia and inflammation, two common features of CKD have been found to alter DNA methylation [11, 19]. Elevated levels of homocysteine affect the DNA methylation equilibrium through increased concentrations of the DNA methyltransferase inhibitor S-Adenosylhomocysteine (SAH). Thus, DNA hypomethylation of peripheral blood cells was found in a small cohort of haemodialysis patients with hyperhomocysteinaemia [20]. Persistent inflammation, on the other hand, is associated with considerably lower levels of homocysteine in the context of uraemia [21]. Consequently, this common feature is linked to hypermethylation rather than hypomethylation [22]. In *in vitro* studies, IL-6 induced

hypermethylation, possibly through regulation of a DNA methyltransferase gene [23, 24]. It is well acknowledged that persistent inflammation is a risk factor for poor outcome in CKD patients [25]. However, it has been demonstrated by our group that global DNA hypermethylation is significantly associated with both all-cause and cardiovascular mortality even following the adjustment for inflammation [11].

Global DNA methylation status seems to be unaffected by CKD itself, as has been shown in two cohorts of predialysis subjects [11, 26]. However, experimental studies demonstrate that local, gene-specific alterations in DNA methylation may play an important role in the pathogenesis of CKD. Bechtel *et al.* [27] showed that hypermethylation of a gene encoding an inhibitor of the Ras oncoprotein (*RASALI*) was associated with the perpetuation of fibroblast activation and fibrogenesis in the kidney. Accumulating evidence suggests that RNA interference is highly important for the development and progression of renal disease [28]. In mice lacking functional miRNAs in podocytes, significant proteinuria develops within two weeks after birth, which is followed by rapid progression of glomerular and tubular injury, and eventually death after one month [29]. Expression of the slit diaphragm proteins, nephrin and podocin, has been found to be decreased in these animals [29]. Similarly, miRNA disruption in mouse podocytes results in podocyte effacement, vacuolization, and hypertrophy, often with crescent formation [30]. In another study, a decrease in podocyte miRNA generation caused podocyte apoptosis and depletion, mesangial expansion, capillary dilation, and glomerulosclerosis [31].

As DNA methylation controls the expression of transporters of amino acids [32], organic cations [33] and uric acid [34], tubular transport of solutes has also been found to be regulated by epigenetic modifications. A low number of nephrons ('nephron underdosing') has been implicated in the pathogenesis of proteinuria, hypertension and CKD [35]. Ritz *et al.* [36] postulate that epigenetics play a distinct role in this phenomenon through mechanisms which involve modifications of gene expression by altered DNA methylation and histone acetylation as well as by allocation of stem cells.

Epigenetics following kidney transplantation

Kidney transplantation remains the best method of renal replacement therapy, both in terms of the quality of life, and patient outcome. Unfortunately, the majority of transplanted patients have to return to dialysis after some time due to chronic graft failure. There are numerous causes of chronic graft insufficiency, not all of which have been thoroughly elucidated. Currently, it is becoming apparent that epigenetic alterations may play some part in the worsening of graft function. Acute rejections are among the risk factors for chronic graft

failure. Therefore, it is of interest that as much as 20 different miRNAs have been identified to be differently expressed in acute rejection after renal transplantation [37]. Chronic graft nephropathy can also be due to prolonged cold ischaemia time [38]. Experimental studies have provided evidence for the role of aberrant DNA methylation in the course of ischaemia-reperfusion injury that might contribute to gene deregulation posttransplant, influencing the development of chronic nephropathy [39].

Epigenetics and cancerogenesis

Kidney cancer is currently the 9th most common cancer in Europe, and the incidence rates for renal cell cancer have been rising steadily over the past three decades [40]. Although numerous polymorphisms and mutations involved in the pathogenesis of renal cell cancer have been identified, an increasing number of studies underline the role of epigenetic modifications in this process. The prognosis of renal cell cancer depends on early detection of the tumor. However, clinical signs and symptoms are often not useful in making an early diagnosis of this cancer, since the classic symptoms: pain, haematuria, and palpable flank mass are usually associated with advanced stage of the disease. Currently, several genes have been confirmed to show frequent promoter region methylation in primary renal cell cancer samples [41–43]. Arai *et al.* [44] demonstrated that alterations of DNA methylation in the precancerous kidney cortex tissue might generate more malignant renal cancer cells and determine patient outcome. Currently, it is becoming acknowledged that it is the interplay between genetics and epigenetics that leads to tumour genesis and the progression of cancer [40]. This interplay have recently been elegantly demonstrated by Dalglish *et al.* [45] who identified mutations in genes that encode enzymes, which demethylate or methylate key lysine residues of histones. Discovered mutations lead to modification of the methylation status of these lysine residues, and consequently alter chromatin structure and transcriptional control. They have been found to be significantly associated with the occurrence of renal cell cancer [45].

Obviously, the potential use of epigenetic modifications in diagnosing renal cell cancer is still in its infancy. However, it seems plausible that epigenetic markers of renal cell cancer detected in urine and/or serum could, in the near future, offer a noninvasive method for early diagnosis, increasing the chance of successful treatment. As epigenetic changes are potentially modifiable, therapeutical interventions through reversal of epigenetic gene silencing could even present an alternative for surgical treatment.

Cancerogenesis is also a serious issue in patients after renal transplantation. The incidence of cancer in these patients reaches 20% during 10 years after transplantation [46]. The most frequent tumours are non-melanoma skin

cancers. Epigenetic mechanisms can be involved in the pathogenesis of these neoplasms as well. Laing *et al.* [47] demonstrated a statistically significant difference between global methylation levels of squamous cell carcinoma and adjacent non-neoplastic skin. Squamous cell carcinoma turned out to be profoundly hypomethylated.

Conclusions

Our understanding of the roles played by epigenetic modifications in CKD remains in its infancy. Thus, further studies are needed to better understand the associations of aberrant DNA methylation, histone modifications, RNA interference and the development and progression of kidney diseases. As epigenetic modifications are potentially reversible, the effects of various epigenetic interventions on outcome in CKD is an intriguing area for future studies.

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Резиме

УЛОГАТА НА ЕПИГЕНЕТИКАТА КАЈ БОЛЕСТИ НА БУБРЕЗИТЕ

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Епигенетиката е иследување на промените во експресијата на гените којашто се случува без промени во секвенцијата на ДНК. Овие реверзибилни модификации ги вклучуваат метилацијата на ДНК, ацети-
лацијата на хистони и интерференцијата на РНК. Епигенетските проме-

ни се фундаментални за физиолошките процеси коишто ги регулираат активностите на генот; затоа, овие промени се изменуваат во одговор на факторите на околината или состојби на болест, но во многу прилики, исто така, се верува дека придонесуваат за појавата и прогресијата (напредувањето) на болеста. Досега, студиите за епигеномот се ретки во нефрологијата, меѓутоа, постои податок дека во уремична средина, неколку карактеристики како инфламацијата, дислипидемијата, хиперхомуцистинемијата, оксидативниот стрес, како и дефицит на витамини и исхраната може да дејствуваат на епигеномот и да влијаат на исходот на пациентот. Оваа статија го опишува сегашното знаење за епигенетските алтерации во текот на различните состојби на бубрежните болести. Иако науката во епигенетиката е сè уште во нејзиниот почеток, таа може да помогне да се разјаснат почетните механизми во уремијата и да овозможи да се развијат нови стратегии на лекување.

Клучни зборови: епигенетика, бубрежна болест, секвенца на ДНК, метилација на ДНА, ацетилација на хистони, интерференција на РНК.

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