Epigenetics in CKD
Rationale for BET protein inhibition, an emerging therapeutic mechanism in renal disease and CVD

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DNA-methylation

In Health

- Development and differentiation of cells
- Genomic imprinting
- X chromosome inactivation

In Disease

- Carcinogenesis: DNMT inhibitors approved for treatment of myelodysplastic syndromes
- Hyper- hypomethylated genes associated with kidney development, CKD & progression, DN and dialysis induced changes

Ageing – the epigenetic clock

- A measure of biological age: ageing is associated with DNA hypermethylation within CpG islands and hypomethylation outside
- Individuals with a higher biological than chronological age are at an increased risk for all-cause mortality
- CKD patients are generally prematurely aged - study of the impact of epigenetics on accelerated biological ageing

Lam K et al. 2016
Five major families of histones: H1/H5 (linkers), H2A, H2B, H3 and H4 (core histones)
The Histone Code

- Acetylation/deacetylation
- Methylation/demethylation
- Phosphorylation
- Ubiquitination
- Sumoylation
- Citrullination

**Writers**
- Histone acetyltransferases (HATs)
- Histone methyltransferases (HMTs)

**Erasers**
- Histone deacetylases (HDACs)
- Histone demethylases, (HDMs)

**Readers**
- Chromodomain proteins
- Bromodomain proteins

Less condensed – more accessible to transcription factors

More condensed – less accessible to transcription factors
RNA-Methylation and RNA Editing

RNA-methylation

- Occurs in transferRNAs, ribosomal RNA, messenger RNAs, microRNAs, small nuclear RNAs, small nucleolar RNAs and viral RNAs
- Regulates many biological processes, including RNA stability and translation
- Involved in determining circadian rythm by regulating circadian clock genes
- Plays a role in immune function – dysregulation contributes to tissue damage and auto-antibody formation in SLE

RNA-editing

- Alters the nucleotide sequence in coding and non-coding RNAs
- Adenosine-to-Inosine conversion (A-to-I) most common.
- Catalysed by enzymes in the adenosine deaminase activity on RNA (ADAR) family
- Disturbed ADAR-activity in vascular disease, cancers, neurological and autoimmune disorders, metabolic diseases and viral infections

Slotkin W and Nishikura K 2013
Non-Coding RNAs

MicroRNAs (20-24 nucleotides)
- Negative regulators by degrading target mRNAs and/or inhibiting protein translation
- Highly pleiotropic and cell type specific; >1900 miRNAs identified in the human genome (www.miRbase.org)
- Many miRNAs have isoforms (isomiRs), sometimes due to A-to-I editing

In disease:
- Circulating levels of particular miRNAs, e.g. miR-143, miR-145, miR-150 and miR-223, were significantly higher in CKD patients compared to controls
- In the kidney, expression levels of miR-125a-5p, miR-125b and miR-217 have been associated with regulation of biological age and health span.
- Hyperphosphatemia induces VSMC calcification mediated by miR-29, miR-133b and miR-211. Therapeutic targets of VC?
- Miravirsen (hepatitis C) is the first miRNA-targeting drug (miR-122) entering a phase II clinical trial
Suggested links between alterations in epigenetic regulation and cellular and physiological homeostasis in the uraemic milieu

Writers, Erasers and Readers

**Writers**
- DNA methyltransferases (DNMTs)
- Histone acetyltransferases (HATs)
- Histone methyltransferases (HMTs)

**Erasers**
- Histone deacetylases (HDACs)
- Histone demethylases (HDMs)

**Readers**
- Chromodomain proteins
- Bromodomain proteins
- Bromodomain and extraterminal family (BETs)

- The Epigenetic code refers to secondary modifications to chromatin components that regulate its activity
- Transcription is regulated by addition, removal or recognition of these modifications (writers, erasers, readers)
- Acetylation is associated with active transcriptional regions of chromatin
- BET (Bromodomain and Extraterminal Domain) proteins bind to acetylated lysines on histones and recruit additional transcription factors
A bromodomain is an \( \approx 110 \) amino acid protein domain that recognizes and bind to acetylated lysine residues on histones \( \rightarrow \) activates gene expression

Dysregulated in several disease states, such as cancer, autoimmune and vascular diseases

Bromodomain and extraterminal domain (BET) family including BRD2, BRD3, BRD4, and BRDT

Involved in normal cellular processes, such as proliferation, apoptosis and transcription

Dysregulation \( \rightarrow \) pathological gene expression of oncogenes \( \rightarrow \) development aggressive tumours

Drivers of inflammation, e.g in atherosclerosis, sepsis and autoimmune diseases

Involved in heart failure and hypertrophy

The name "bromodomain" is derived from the relationship of this domain with Brahma (in drosophila) and is unrelated to the chemical element bromine.
**BET Protein Inhibitors**

- **BET protein inhibitors** are a class of drugs with anti-cancer, immunosuppressive, anti-inflammatory and other effects currently in clinical trials. These molecules reversibly bind the bromodomains of BRD2, BRD3, BRD4, and BRDT, preventing the BET proteins from binding to the acetylated histones and blocking transcription.

- **Apabetalone (RVX-208):** oral, small molecule BET inhibitor with specificity for BD2 of BRD4.


- Increased HDL-C, as well as ApoAI and large HDL particles, both believed to be important factors in enhancing reverse cholesterol transport (RCT). Lowered the incidence of Major Adverse Cardiac Events (MACE).

- In a CKD subgroup, Alkaline Phosphatase (ALP) was decreased (associated with CVD risk in diabetes and CKD; predictor of all cause mortality) and eGFR was increased.

- Proteomics study in CKD4 &CKD5 patients: a single dose of RVX-208 downregulated proteins and pathways involved in endothelial dysfunction, atherosclerosis, vascular inflammation & calcification, fibrosis, haemostasis and chronic inflammation.
Apabetalone is a bromodomain extra-terminal (BET) protein inhibitor which can regulate the expression of genes and restore the function of pathways underlying the pathogenesis of cardiovascular disease.

- Reductions in components and the function of the complement cascade
  - Wasiak S et al. 2017
  - Wasiak S et al. 2018

- Reductions in the components of the coagulation cascade
  - Wasiak S et al. 2018

- Reductions in mediators that promote calcium deposition in the vasculature
  - Wasiak S et al. 2018

- Reductions in mediators that promote inflammation of the vasculature
  - Wasiak S et al. 2018

- Increased ApoA-I, positive effect on lipid content of HDL
  - Kulikowski e et al. 2018

- Delayed and reduced oral glucose absorption and endogenous production

Reduced incidence of Major Adverse Cardiac Events
Phase 3 clinical trial BETonMACE initiated in 2015: 2400 patients enrolled

Effect of Apabetalone on Major Adverse Cardiac Events (MACE) in high-risk CVD patients with T2D and low high-density lipoprotein (HDL).

Patients with CKD and neurodegenerative diseases

Renal function will be followed in the subgroup of patients with CKD

Neurological function assessed in patients aged ≥70 by the Montreal Cognitive Assessment (MoCA).

In pipeline:
- Phase 2 BETonRenal
- Phase 2 Fabry Disease
The Epigenetic Code – Regulating Gene activity

- Definition 1: Stably heritable traits (through DNA replication and cell division), which are mediated by DNA cytosine methylation and post-translational modifications of histones - the two most well studied epigenetic mechanisms.

- Definition 2: RNA methylation and non-coding RNAs, such as small non-coding RNAs (microRNAs (miRNAs), piwi-interacting RNAs (piRNAs) and short interfering RNAs (siRNAs)) and long non-coding RNAs (lncRNAs).

- Cross-talk between histone modifications, DNA-methylation and non-coding RNAs; almost infinite number of ways to calibrate the genetic code and regulate gene activity.

- Challenge: The epigenetic landscape varies between tissues and cell populations, over time and in health and disease difficult to study and modulate with specificity (unwanted/beneficial side effects).

- Instead of the "One target – One drug" model, epigenetic drugs open for novel therapeutic possibilities to target several disease causing proteins and associated complications simultaneously.