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Global Spotlights

Epigenetic control of vascular endothelial function revealed by multi-omics

Hywel Dunn-Davies[1], Tatiana Dudnakova[1], Andrew H. Baker [2], and Tijana Mitić [2]*

[1]Wellcome Centre for Cell Biology, University of Edinburgh, Michael Swann Building Max Born Crescent, King's Buildings, Edinburgh, EH9 3BF, UK; and [2]Centre for Cardiovascular Science, The Queen's Medical Research Institute (QMRI), University of Edinburgh, BioQuarter, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK

Endothelial cell (EC) dysfunction is a common mechanism that can drive, and contribute substantially, to a broad range of cardiovascular diseases (CVDs). Endothelial cells are a mechanical and physical barrier between the circulating blood and adjacent tissues. In response to signalling cues, ECs undergo dynamic changes, for example reshaping their structure through intricate cytoskeletal re-arrangements. Such perturbations lead to endothelial dysfunction and leukocyte infiltration, which are associated with the development of CVD-like atherosclerosis.[1]

Many cell surface ligands and transmembrane receptors orchestrate the delicate processes of cellular adhesion in developing and mature endothelium. The tight adherence of ECs to the extracellular matrix involves integrin-based adhesion complexes that connect to the actin cytoskeleton.[2] Understanding these regulatory mechanisms that drive the initiation of EC dysfunction can improve our understanding of the disease pathogenesis to develop new therapeutic approaches. Such mechanisms are often complex and require unbiased approaches to identify them.

In the last decade, much attention has been paid to the non-coding RNA (ncRNA) transcriptome. Within this, long ncRNAs (lncRNAs), which are 200 nt long transcripts that do not code for proteins, have received intense scrutiny. Yet, the vast numbers of lncRNAs in any given cell and their diverse functions (or indeed assignment as simply 'noise') make them challenging to understand. However, RNA sequencing can measure lncRNA expression to provide an initial understanding of their cellular levels and perturbation to associate with a particular phenotype. The cellular location in which lncRNAs are found also defines their potential function. In the nucleus, many lncRNAs play a role in epigenetics by recruiting transcription factors or chromatin-modifying complexes onto chromatin, or in structuring, supporting, or impeding protein complexes.[2,3] The use of various chromatin immunoprecipitation techniques with sequencing has helped to identify lncRNAs that have a so-called epigenetic function. Further implementation of multi-omics and associated bioinformatics pipelines can further help identify genomic sequences that are targeted by lncRNA.[3] For example, employing **c**hromatin **i**solation by **R**NA **p**urification with sequencing (ChIRP-seq) has already opened exciting avenues for the discovery of lncRNA targets unveiling an intricate network of genomic regulations.[4] Leveraging large-scale genome-wide association studies datasets in a multi-omic framework can further verify the molecular targets of individual lncRNAs and help identify potential disease traits or associated non-coding *loci*, as outlined in *Figure 1A*.[5]

It is widely known that many nuclear lncRNAs directly bind the polycomb repressive complex 2 (PRC2) and its catalytic subunit, Enhancer of zeste homologue 2 (EZH2), which writes di-methylation and tri-methylation of histone H3 Lys27. This phenomenon can, for example, lead to transcriptional silencing of genes in the heart.[6] Therapeutically, inhibitors that target EZH2 methyltransferase activity have already proven beneficial in models of endothelial dysfunction, atherosclerosis, and aortic aneurysm.[6,7] Moreover, the current development of highly selective chemical modalities, such as proteolysis targeting chimeras (PROTACs®), can disrupt PRC2 structure.[8] PROTACs® utilize the ubiquitin–proteasome system as the natural cellular protein degradation system, to break a specific protein down, instead of binding to it like traditional small molecules do. Theoretically, such novel approaches in drug development for the discovery of PRC2-controlled pathways could be used for the treatment of cardiovascular conditions.

Studying EZH2 interactors in endothelial cell dysfunction

To elucidate the consequences of EZH2 binding to EC RNA, we captured transcriptome-wide RNA and RNA–RNA interactions with EZH2 in primary ECs. This unbiased approach was enabled through cross-linking and sequencing employing an innovative technique called formaldehyde/UV-assisted cross-linking ligation and sequencing of hybrids (FLASH). We generated the data set, in collaboration with Prof. David Tollervey, who pioneered this technique in his laboratory at

* Corresponding author. Email: tijana.mitic@ed.ac.uk

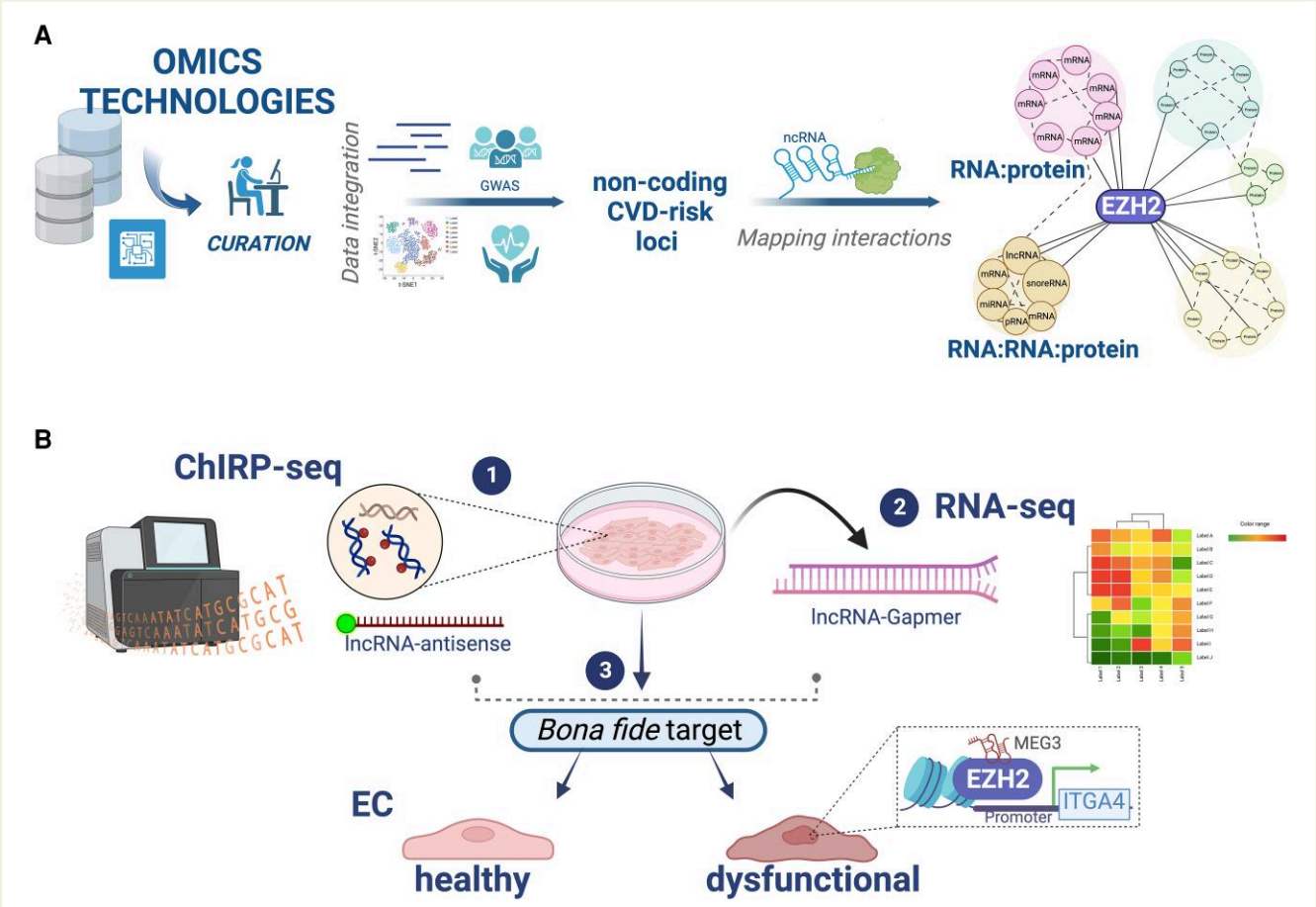

Figure 1 (*A*) Molecular effector mechanisms behind many non-coding RNAs exhibit a considerable diversity. Leveraging the multi-omic databases ('bulk' and 'single' transcriptomic and genomic signals) together with the genome-wide association studies can be a powerful tool to explore novel non-coding cardiovascular disease-risk loci and their cellular setting. By integrating the data sets, we can map the regulatory interactions between non-coding RNAs and transcriptional regulators. Like so, exploring the RNA binding capacity of EZH2 protein has revealed direct RNA targets and sites of RNA:protein and RNA:RNA:protein interaction. (*B*) Use of chromatin isolation by RNA purification by employing the biotinylated antisense probes against long non-coding RNA of interest allows the discovery of associated genomic targets for same long non-coding RNA (1). Simultaneous use of bulk RNA-sequencing of long non-coding RNA-deficient cells can ultimately help discern the *bona fide* long non-coding RNA targets in endothelial cells (2) by integrating the two data sets from approaches in 1 and 2. We already reported an intricate network of genomic and transcriptional regulation of ITGA4 in endothelial cells. GWAS, genome-wide association studies; CVD, cardiovascular disease; ncRNA, non-coding RNA; EZH2, Enhancer of zeste homologue 2; ChIRP-seq, chromatin isolation by RNA purification; RNA-seq, RNA sequencing; EC, endothelial cell; lncRNA, long non-coding RNA; MEG3, maternally expressed gene 3; ITGA4, integrin subunit alpha 4 (image created with BioRender.com).

the University of Edinburgh.[4] This has prompted a fruitful collaboration between the Wellcome Trust Bioinformatics Core and the British Heart Foundation Centre of Excellence at the Centre for Cardiovascular Science, University of Edinburgh. This research has revealed two endothelial-specific isoforms of the lncRNA called 'maternally expressed gene 3' (MEG3).[9] It emerged that EZH2 directly binds to the RNA–RNA duplexes formed by the MEG3 in ECs.[4]

It remained unknown how exactly MEG3 and EZH2 'collaborate' in this intricate genetic symphony in ECs. To address this, we generated an endothelial MEG3-genome binding data set from ChIRP-seq.[4] The omic analysis used to discern *bona fide* MEG3-targeted regulatory pathways in ECs is described in *Figure 1B*. Interestingly, one target of MEG3:EZH2 complex is the integrin subunit alpha 4 (*ITGA4*) EC receptor that is involved in the angiogenesis response and a pivotal player in cellular adhesion and cytoskeletal rearrangement,[4,9] thus essential for maintenance of functional endothelium in vascular health. Consequently, we proposed that MEG3 guides EZH2 onto a regulatory region of *ITGA4*, locking it in a repressed state. Deletion of MEG3 or pharmacological inhibition of EZH2 weakened the interaction with *ITGA4*, which led to an increased *ITGA4* expression and improvement in EC function.[4]

The broader impact on endothelial cell homeostasis and disease

Exploring the fundamental molecular mechanisms that defined lncRNA use in an endothelial perspective is intriguing, as it holds the potential to reveal disease drivers. Multi-omic approaches that progressively deepened this understanding have proven pivotal in unravelling molecular effectors. Intricate and complex partnerships of lncRNA with proteins like EZH2 give us a far better understanding of the delicate epigenetic alterations that occur during injury and dysfunction, like in the diseases

associated with clinical vascular leak. Collectively, we then reach the longer-term possibility to re-evaluate and re-purpose current FDA (food and drug administration)-approved treatments for vascular diseases or create new targeted approaches, which could selectively target a defined epigenetic interaction, thus preventing or potentially reversing the endothelial dysfunction in CVD.

Declarations

Disclosure of Interest

All authors declare no disclosure of interest for this contribution.

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Patients on ENTRESTO experience fewer hospitalisations, reduced risk of CV death and improved QoL versus ACEi (enalapril)*[3–7]

QoL based on post hoc analysis[6,7]

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Current, expert-led ESC guidelines recommend ENTRESTO as a first-line treatment option for eligible patients with symptomatic chronic HFrEF in combination with a BB, SGLT2i and MRA[8]

~2% **of the NHS budget is spent on HF†[9,10]**

~70% **of the cost of HF to the NHS is due to hospitalisation[9]**

Versus ACEi (enalapril), at a median follow-up of 27 months, **ENTRESTO** significantly **reduced the risk** of:[‡3]

Composite of death from CV causes or first hospitalisation for worsening HF	Death from CV causes	First hospitalisation for worsening HF
20% RRR (ARR=4.7%; p<0.001)	20% RRR (ARR=3.1%; p<0.001)	21% RRR (ARR=2.8%; p<0.001)

Starting ENTRESTO first-line could add 1 to 2 years to patients' lives vs ACEi[4]

Based on actuarial estimates from the PARADIGM-HF trial, and assuming that protective effects of ENTRESTO remain consistent with long-term use; extrapolated from available short-term follow-up data. Results were found in patients who were 45–75 years of age.[4]

The most commonly reported adverse reactions with ENTRESTO were hypotension (17.6%), hyperkalaemia (11.6%) and renal impairment (10.1%); angioedema was reported in patients treated with ENTRESTO (0.5%; uncommon).[1,2]

For further safety information, please refer to the Summary of Product Characteristics[1,2]

ACEi, angiotensin converting enzyme inhibitor; ARR, absolute risk reduction; BB, beta blocker; CV, cardiovascular; DHSC, Department of Health and Social Care; EF, ejection fraction; ESC, European Society of Cardiology; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; MRA, mineralocorticoid receptor agonist; QoL, quality of life; RCT, randomised controlled trial; RR, risk reduction; SGLT2i, sodium-glucose cotransporter 2.

*PARADIGM HF (N=8,442) was a double-blind RCT of patients with class II, III or IV HF and an EF of ≤40% randomised to receive either ENTRESTO (200 mg twice daily) or enalapril (10 mg twice daily) in addition to recommended therapy. Primary outcome was a composite of death from CV causes or hospitalisation for HF[1]; †NHS budget 2020–2021 based on DHSC departmental expenditure limit of £130.38 billion[10]; ‡N=8,399.

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