Tackling the complexity of CKD-associated cardiovascular disease

Chapter 8

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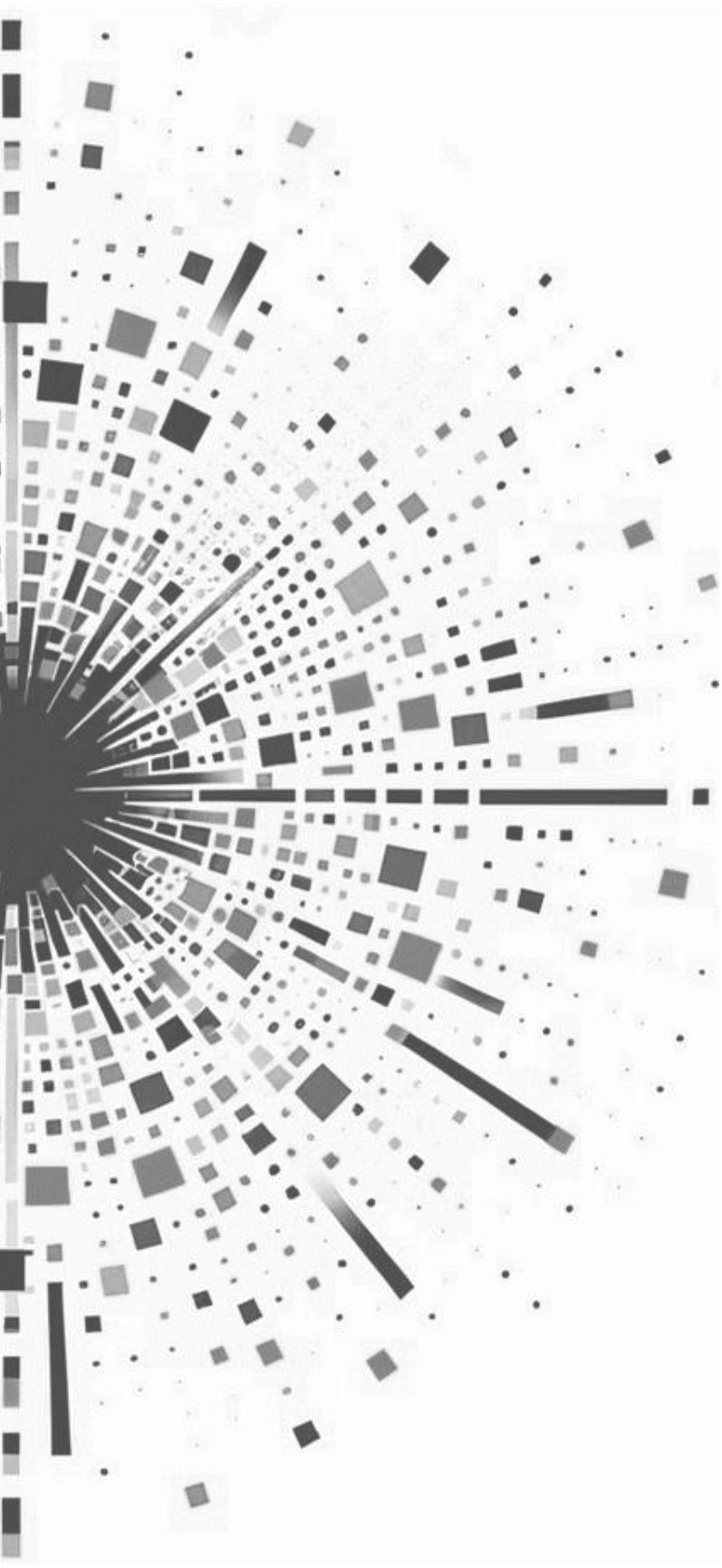

Unmet need in dialysis research

This thesis is dedicated to studying the mechanisms underlying cardiorenal syndrome type IV, which describes cardiovascular complications caused by renal insufficiency[1]. Increased cardiovascular morbidity is, amongst others, associated with elevated concentrations of the uremic milieu in patients with chronic kidney disease (CKD)[2]. In **Chapter 2** of this thesis, we provide evidence that there is currently no universally suitable technique for reduction of all known 33 protein-bound uremic toxins (PBUTs), yet several approaches showed promising results for certain compounds, e.g. supplementation of haemodialysis patients with oral adsorbent AST-120 (Kremezin®) for reduction of indoxyl sulphate and *p*-cresyl sulphate in long-term trials. The systematic analysis made it clear that, due to the high heterogeneity of PBUTs, it is unlikely to achieve optimal reduction of all the compounds with a single therapy. Our work identifies the need for bigger longitudinal multicentre trials to study the effectiveness of combination therapies targeting compounds of different nature. Additionally, the review demonstrates the evident lack of research for reduction of certain PBUTs, e.g. 2-methoxyresorcinol, hydroquinone, putrescine and others, and serves as a helpful starting point for future studies targeting management of these understudied toxins. In a healthy kidney PBUTs are removed predominantly though tubular secretion, which is challenging to replicate with conventional dialysis. Thus, preservation of residual kidney function by, for instance, controlling blood pressure and minimizing use of nephrotoxic agents (e.g. radiocontrast dyes), as well as dietary interventions such as restriction of protein and increase of fibre and complex carbohydrates intake, can help to deceleration CKD progression and improve patients' survival[3,4].

Role of post-translational protein modifications

Another factor that may link CKD to cardiovascular disease (CVD) risk increment is protein carbamylation, one of the first identified post translational protein modification (PTM) shown to be associated with uremia[5,6]. Its major role in cardiovascular disease was first brought to light by the group of Prof. S.L. Hazen with their work published in 2007 in Nature Medicine showing protein carbamylation resulting from myeloperoxidase activity in atherosclerotic plaque and blood carbamylation levels being predictive for patient death[7]. Leaving aside anti-carbamylation antibodies that are recently becoming recognized as a promising biomarker for rheumatoid arthritis and other diseases[8–10], carbamylation research received modest interest for the past decade with 20-30 articles per year. With our work in **Chapter 3**, we show that this type of protein modification is worth attention demonstrating its abundance in late stage hemorrhaged plaques of patients with kidney insufficiency and association with foam cells. CKD patients are known to have increased burden of atherosclerosis with plaques showing elevated risk of rupture[11,12]. It remains to be determined if plaque carbamylation is causally related to its vulnerability in patients with CKD. Additionally, we have seen differential cellular effects of carbLDL uptake by human macrophages compared to oxLDL uptake. This raises many questions such as: what the exact mechanism is of carbLDL trafficking, processing, and

storage by the cells compared to that of oxLDL; to which extent these processes are affected by the severity of the modification, and if they can be interfered with or, on the contrary, taken advantage of to influence the cells' faith and, as a result, plaque progression. Moreover, it is known that LDL proteins are not the only targets of carbamylation. In our results, a big portion of the extracellular matrix material in plaque, e.g. collagens, were also seen to stain positive for carb-lys. How this affects activity of the surrounding cells in the plaque as well as biomechanical properties of the protein itself and, consequently, plaque phenotype, remains to be determined. Finally, our confocal images revealed a strong nuclear carb-lys signal present in human macrophages. The mechanisms and significance of this nuclear carbamylation are yet to be dissected but may potentially hold a key to yet unknown gene expression regulation processes. To summarise, **Chapter 3** of this thesis highlights the necessity for future PTM studies and unveils possibilities for new discoveries of carbamylation-related cellular mechanisms.

PTM formation is not only an adverse process. In fact, practically all proteins require PTMs for proper functioning. In a mini review in **Chapter 4** we highlight the importance of post-translational cleavage of Klotho protein for its systemic function pointing out the difference between various forms of secreted Klotho, e.g. KL1, KL2, and full-length proteins. As can be seen from the review, higher levels of soluble Klotho in human were shown to be associated with lower risk of kidney disease while beneficial effects of its administration or overexpression have been plentifully demonstrated in mice, e.g. reduction of renal injury, improved recovery and survival, as well as prevention of calcification. However, no distinction has been made between the roles of full vs shorter Klotho forms while no information is available on the functions of KL2 thus far due to the lack of specific antibodies. This highlights the need for further studies to elucidate the molecular mechanisms behind generation of Klotho variants and the lack of knowledge regarding their, possibly, distinct functions. Better understanding of soluble Klotho generation and functioning could allow future drug discovery and generation of more precise therapies to target calcification and age-related complications.

Novel drug targets in atherosclerosis

The identification of novel drug targets holds paramount importance in the development of efficacious therapeutic interventions across various pathological conditions. In this context, computational methodologies, such as weighted gene co-expression network analysis, offer a transformative solution allowing researchers to uncover hidden relationships between genes and their functions and providing insights into the molecular mechanisms underlying disease. Notably, our discovery of CDK5 as a previously unrecognized contributor to calcification (**Chapter 5**), underscores this potential. By analysing large sets of genetic data, macrophage CDK5 emerged as a central regulator of a gene program linked to calcification. Indeed, its deficiency led to reduced vascular calcification possibly by dampening inflammation *in vitro* and *in vivo*. This demonstrates how computational methods can reveal novel targets that

might have been overlooked using traditional approaches. The comprehension of the intricate molecular interactions allows discovery of innovative therapeutics tailored towards these new factors, thereby promoting development of enhanced and targeted interventions for cardiovascular disease and accompanying morbidities. Our work shows that myeloid-specific inhibition of CDK5 would potentially reduce plaque burden through decreasing inflammation and, subsequently, calcification.

However, a few hurdles remain to be addressed. Firs, to the best of our knowledge, there is only one specific CDK5 inhibitor described in the literature so far that still requires testing *in vivo*[13] while there are currently no CDK5-specific inhibitors being tested in clinical trials. Additionally, the therapeutic benefits of CDK5 inhibitors for atherosclerosis alleviation need to be investigated, as CDK5 is ubiquitously expressed by many other cell types, e.g. pancreatic β cells and neurons, and shown to play a vital role in neuronal development and cell survival[14,15]. Thus, a more targeted approach might be required to avoid possible side effects of systemic CDK5 inhibition. This could be achieved through, for example, implementation of antibody-drug conjugate technology for plaque macrophage targeting. However, such system remains to be developed. It would be also interesting to study the downstream targets of CDK5 in the plaque to possibly reveal a more precise targets for attenuation of plaque inflammation and calcification. Taken together, our study sheds light on possibilities for novel drug targets for treatment of atherosclerosis.

Importance of scientific collaboration

In the environment of high competition and scares financial resources, researchers should be encouraged and incentivised to put aside their personal interests and engage in transparent exchange of knowledge and discoveries for the sake of societal progress. Hence, this work would have not been possible without collaborative effort from several parties. With this thesis we established diverse collaboration network between University of Maastricht and a number of other of universities in Germany, UK, Sweden, and Italy. Projects initiated with this work continue to develop beyond the scope of the thesis bringing a possibility of promising high impact publications and valuable scientific contribution in the future.

Taken together, this thesis broadens our understanding of complex processes of cardiorenal syndrome opening up opportunities for future drug discovery and personalized therapies to reduce the burden of CDK-associated cardiovascular disease.

References

1. Rangaswami, J. *et al.* Cardiorenal Syndrome: Classification, Pathophysiology, Diagnosis, and Treatment Strategies: A Scientific Statement From the American Heart Association. *Circulation* **139**, E840–E878 (2019).
2. El Chamieh, C., Liabeuf, S. & Massy, Z. Uremic Toxins and Cardiovascular Risk in Chronic Kidney Disease: What Have We Learned Recently beyond the Past Findings? *Toxins (Basel)* **14**, (2022).
3. Faria, M. & de Pinho, M. N. Challenges of reducing protein-bound uremic toxin levels in chronic kidney disease and end stage renal disease. *Transl Res* **229**, 115–134 (2021).
4. Mathew, A. T., Fishbane, S., Obi, Y. & Kalantar-Zadeh, K. Preservation of Residual Kidney Function in Hemodialysis Patients: Reviving an Old Concept for Contemporary Practice. *Kidney Int* **90**, 262 (2016).
5. Stark, G. R., Stein, W. H. & Moore, S. Reactions of the Cyanate Present in Aqueous Urea with Amino Acids and Proteins. *J Biol Chem* **235**, (1960).
6. Erill, S., Calvo, R. & Carlos, R. Plasma protein carbamylation and decreased acidic drug protein binding in uremia. *Clin Pharmacol Ther* **27**, 612–618 (1980).
7. Wang, Z. *et al.* Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med* **13**, 1176–1184 (2007).
8. Dong, R. *et al.* Distribution and clinical significance of anti-carbamylation protein antibodies in rheumatological diseases among the Chinese Han population. *Front Immunol* **14**, (2023).
9. Calixto, O. J. *et al.* Posttranslational modifications in psoriatic arthritis: A systematic literature review. *Autoimmun Rev* **22**, (2023).
10. Liem, S. I. E. *et al.* Anti-carbamylated protein antibodies in systemic sclerosis. *Arthritis Res Ther* **25**, (2023).
11. Valdivielso, J. M. *et al.* Atherosclerosis in Chronic Kidney Disease. *Arterioscler Thromb Vasc Biol* **39**, 1938–1966 (2019).
12. Pelisek, J. *et al.* Impact of chronic kidney disease on carotid plaque vulnerability. *J Vasc Surg* **54**, 1643–1649 (2011).
13. Daniels, M. H. *et al.* Discovery and Optimization of Highly Selective Inhibitors of CDK5. *J Med Chem* **65**, 3575–3596 (2022).
14. Kanungo, J., Zheng, Y. L., Amin, N. D. & Pant, H. C. Targeting Cdk5 Activity in Neuronal Degeneration and Regeneration. *Cell Mol Neurobiol* **29**, 1073 (2009).
15. Cheung, Z. H., Gong, K. & Ip, N. Y. Cyclin-Dependent Kinase 5 Supports Neuronal Survival through Phosphorylation of Bcl-2. *The Journal of Neuroscience* **28**, 4872 (2008).