The effect of intra- and extracellular challenges on cellular responses in atherosclerosis

Chapter 10

Impact

Cardiovascular disease (CVD) is globally still the number one cause of death, with a number as high as 17.9 million deaths in 2017.[1] The global burden of CVD is not limited to health issues, but also affects economic status. Costs within the European Union for CVD and its clinical manifestations were estimated at €210 billion a year, in 2015.[2] One of the main causes of CVD is atherosclerosis, which is affected by numerous risk factors such as diet, sedentary lifestyle and smoking. In this thesis, we investigated numerous cellular stressors and how they affected atherosclerosis development. In this chapter, we will put these findings in scientific and social perspective.

As the numbers above already state, improvement of CVD treatment is highly necessary. Treatment options nowadays include lipid lowering drugs, blood pressure medication or invasive removal of the atherosclerotic plaques by surgery. By investigating different intra- and extracellular challenges in the context of atherosclerosis, we were able to draw conclusions from different environmental states on plaque development. Hypoxia is known to be linked to plaque instability and thus the risk of plaque rupture.[3] Regulation of hypoxia sensors prolyl hydroxylase domain proteins (PHD) 1, 2 and 3 (**Chapter 5**) in immune cells led to different outcomes on atherosclerosis. Here, we show that PHD2 and 3 inhibition might negatively affect atherosclerosis as plaque size was increased, as well as cell death and fibrosis in case of PHD2. Chronic kidney disease patients are already receiving pan-PHD inhibitors for treatment of anemia. This could potential harm them, as they are already at risk for CVD. Cell and PHD-specific inhibition could however be of interest as PHD1 inhibition led to decreased cholesterol levels in mice.[4] Next to PHDs, we also looked into carbonic anhydrase IX (CAIX) which is a pH regulator and hypoxia related enzyme (**Chapter 3**). CAIX could however not be correlated to cardiovascular outcome, nor did it affect immune cell phenotype in relation to atherosclerosis. Hence, we concluded that CAIX itself would not be of interest as a biomarker for CVD. From these findings regarding hypoxia, we can conclude that more research is needed in tailoring hypoxia targeting and lowering plaque vulnerability.

Chaperone mediated autophagy (CMA), a key process in cell homeostasis, was shown to be protective against atherosclerosis (**chapter 2**). Modulation of CMA could therefore be a potential new target in atherosclerosis treatment. Activation can be triggered via endogenous activators such as humanin or retinoic acid antagonists.[5, 6] By activating CMA in atherosclerosis, cell homeostasis in the plaque could be improved leading to a more beneficial plaque environment. Our studies only take into account the effect of CMA activation or inhibition before onset of atherosclerosis, but it would of course be more valuable for the clinic to investigate this when plaques are already present. This would resemble the situation of patients in the clinic, as they only see a doctor when symptoms occur.

We also discuss a relatively new player in atherosclerosis, the fibroblast (**Chapters 6 & 7**). We show the extent heterogeneity of fibroblasts, which could be of interest when targeting them. As of now, little is known about the contribution of fibroblasts to atherosclerosis.

Therefore, further studies would be needed to investigate their exact role. Fluorescent reporter mice could be a good approach for this, as they nicely visualize presence and origin in health and disease. Another option could be to directly target fibroblasts and initiate their depletion in the vascular wall to investigate their function.

A key scientific method that we have used in this dissertation is single cell RNA sequencing (scRNA-seq). scRNA-seq has been emerging in the field of atherosclerosis as it allows researchers to evaluate cells in depth based on gene expression. It has been essential in identifying small subsets present in the vasculature or to map cellular participants in disease.[7-9] By using scRNA-seq in this thesis, we were able to visualize fibroblast heterogeneity in healthy adventitia and could show distinct subsets. In the future, scRNA-seq could potentially be the golden standard when investigating CVD or any other disease. The amount of genetic data and the ability to investigate cell-cell communication could potentially lead to new discoveries in disease mechanisms and hence also possible treatment options.

The broad data presented in this dissertation shows the importance of numerous cellular responses in atherosclerosis. It gives a plethora of possibilities to target CVD and to improve therapeutics in the future

References

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, Alla F, Alvis-Guzman N, Amrock S, Ansari H, Arnlov J, Asayesh H, Atey TM, Avila-Burgos L, Awasthi A, Banerjee A, Barac A, Barnighausen T, Barregard L, Bedi N, Belay Ketema E, Bennett D, Berhe G, Bhutta Z, Bitew S, Carapetis J, Carrero JJ, Malta DC, Castaneda-Orjuela CA, Castillo-Rivas J, Catala-Lopez F, Choi JY, Christensen H, Cirillo M, Cooper L, Jr., Criqui M, Cundiff D, Damasceno A, Dandona L, Dandona R, Davletov K, Dharmaratne S, Dorairaj P, Dubey M, Ehrenkranz R, El Sayed Zaki M, Faraon EJA, Esteghamati A, Farid T, Farvid M, Feigin V, Ding EL, Fowkes G, Gebrehiwot T, Gillum R, Gold A, Gona P, Gupta R, Habtewold TD, Hafezi-Nejad N, Hailu T, Hailu GB, Hankey G, Hassen HY, Abate KH, Havmoeller R, Hay SI, Horino M, Hotez PJ, Jacobsen K, James S, Javanbakht M, Jeemon P, John D, Jonas J, Kalkonde Y, Karimkhani C, Kasaeian A, Khader Y, Khan A, Khang YH, Khera S, Khoja AT, Khubchandani J, Kim D, Kolte D, Kosen S, Krohn KJ, Kumar GA, Kwan GF, Lal DK, Larsson A, Linn S, Lopez A, Lotufo PA, El Razek HMA, Malekzadeh R, Mazidi M, Meier T, Meles KG, Mensah G, Meretoja A, Mezgebe H, Miller T, Mirrakhimov E, Mohammed S, Moran AE, Musa KI, Narula J, Neal B, Ngalesoni F, Nguyen G, Obermeyer CM, Owolabi M, Patton G, Pedro J, Qato D, Qorbani M, Rahimi K, Rai RK, Rawaf S, Ribeiro A, Safiri S, Salomon JA, Santos I, Santric Milicevic M, Sartorius B, Schutte A, Sepanlou S, Shaikh MA, Shin MJ, Shishehbor M, Shore H, Silva DAS, Sobngwi E, Stranges S, Swaminathan S, Tabares-Seisdedos R, Tadele Atnafu N, Tesfay F, Thakur JS, Thrift A, Topor-Madry R, Truelsen T, Tyrovolas S, Ukwaja KN, Uthman O, Vasankari T, Vlassov V, Vollset SE, Wakayo T, Watkins D, Weintraub R, Werdecker A, Westerman R, Wiysonge CS, Wolfe C, Workicho A, Xu G, Yano Y, Yip P, Yonemoto N, Younis M, Yu C, Vos T, Naghavi M, Murray C. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol* 2017;**70**:1-25.
2. Timmis A, Townsend N, Gale CP, Torbica A, Lettino M, Petersen SE, Mossialos EA, Maggioni AP, Kazakiewicz D, May HT, De Smedt D, Flather M, Zuhlke L, Beltrame JF, Huculeci R, Tavazzi L, Hindricks G, Bax J, Casadei B, Achenbach S, Wright L, Vardas P, European Society of C. European Society of Cardiology: Cardiovascular Disease Statistics 2019. *Eur Heart J* 2020;**41**:12-85.
3. Marsch E, Sluimer JC, Daemen MJ. Hypoxia in atherosclerosis and inflammation. *Curr Opin Lipidol* 2013;**24**:393-400.
4. Marsch E, Demandt JA, Theelen TL, Tullemans BM, Wouters K, Boon MR, van Dijk TH, Gijbels MJ, Dubois LJ, Meex SJ, Mazzone M, Hung G, Fisher EA, Biessen EA, Daemen MJ, Rensen PC, Carmeliet P, Groen AK, Sluimer JC. Deficiency of the oxygen sensor prolyl hydroxylase 1 attenuates hypercholesterolaemia, atherosclerosis, and hyperglycaemia. *Eur Heart J* 2016;**37**:2993-2997.
5. Gong Z, Tasset I, Diaz A, Anguiano J, Tas E, Cui L, Kuliawat R, Liu H, Kuhn B, Cuervo AM, Muzumdar R. Humanin is an endogenous activator of chaperone-mediated autophagy. *J Cell Biol* 2018;**217**:635-647.
6. Anguiano J, Garner TP, Mahalingam M, Das BC, Gavathiotis E, Cuervo AM. Chemical modulation of chaperone-mediated autophagy by retinoic acid derivatives. *Nat Chem Biol* 2013;**9**:374-382.

7. Winkels H, Ehinger E, Vassallo M, Buscher K, Dinh HQ, Kobiyama K, Hamers AAJ, Cochain C, Vafadarnejad E, Saliba AE, Zernecke A, Pramod AB, Ghosh AK, Anto Michel N, Hoppe N, Hilgendorf I, Zirlik A, Hedrick CC, Ley K, Wolf D. Atlas of the Immune Cell Repertoire in Mouse Atherosclerosis Defined by Single-Cell RNA-Sequencing and Mass Cytometry. *Circ Res* 2018;**122**:1675-1688.
8. Dobnikar L, Taylor AL, Chappell J, Oldach P, Harman JL, Oerton E, Dzierzak E, Bennett MR, Spivakov M, Jorgensen HF. Disease-relevant transcriptional signatures identified in individual smooth muscle cells from healthy mouse vessels. *Nat Commun* 2018;**9**:4567.
9. Fernandez DM, Rahman AH, Fernandez NF, Chudnovskiy A, Amir ED, Amadori L, Khan NS, Wong CK, Shamailova R, Hill CA, Wang Z, Remark R, Li JR, Pina C, Faries C, Awad AJ, Moss N, Bjorkegren JLM, Kim-Schulze S, Gnjatic S, Ma'ayan A, Mocco J, Faries P, Merad M, Giannarelli C. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med* 2019;**25**:1576-1588.