Healthy aging

Chapter 7

Summary

In this thesis we have attempted to identify the biological processes that lie at the root of the very complex phenomenon of cardiac aging. To reach this goal, we have made use of murine models, which are traditionally the animal models of choice for translational work in the cardiovascular research field. We have used mice that were naturally aged in combination with four accelerated aging mouse models, the latter ones carrying mutations affecting specific pathways commonly regarded as contributors to aging. Additionally, we have reached for a new model, cardiomyocytes derived from human iPSC clones, which is growing increasingly impactful in the cardiovascular field as one of the first human-based experimental platforms.

In **Chapter 3** we have characterized the transcriptomes of hearts from the naturally aged and the four accelerated aged murine models. Resulting from this analysis, we have detected dramatic, model-specific differences between the gene expression profiles of the prematurely aged mouse models. We also observed that naturally aging mice display remarkably stable expression profiles of protein-coding genes and various non-coding RNAs species over time. The overall conclusion we drew from this experimental work is that the mammalian myocardium shows evident transcriptomic stability over time, without any specific process likely underlining the phenomenon of “cardiac aging”.

In **Chapter 4**, we have focused our attention on genomic instability, one specific mechanism commonly regarded as a culprit for senility, as it accumulates random genomic damage, which is generally trademark of tumorigenesis and other pathological conditions that accompanies the passage of time. Specifically, from the RNA- and whole genome DNA-sequencing data of hearts from naturally and prematurely aged mice, we have extrapolated the number of variants accumulating in their transcriptomes and genomes. Our findings indicate that no enrichment in variants is evident in naturally aging murine hearts nor in hearts from the

Lmna$^{G609G/G609G}$ or the G3 *Tert* KO models. Conversely, a substantial accumulation of alterations was only found in in *Ercc1* cardiomyocyte-specific knockout mice, which lack a component of the DNA repair machinery, and in the hearts of *Harlequin* mice, highly sensitive to oxidative stresses. The main conclusion of this study is that the mammalian heart has a remarkable resilience against genomic instability and that defects of the DNA repair machinery is unlikely a cause for "cardiac aging". Overall, the combined observations of **Chapter 3** and **Chapter 4** lead us to challenge the concept of "cardiac aging" or "cardiac senility" as representing merely a byproduct, a consequence, of a variety of age-related comorbidities (e.g. hypertension, vascular pathologies, metabolic syndrome) that largely originate external to the myocardium and are likely to weaken the heart in the elderly.

Finally, in **Chapter 5**, we have reported our first results on one specific circular RNA molecule, circZNF609, which we have found up-regulated both in the hearts of progeroid *Lmna*$^{G609G/G609G}$ knock-in mice and in human cardiomyocytes derived from a hiPSC clone of a patient with Hutchinson Gilford Progeria Syndrome. Based on the level of expression of an array of pluripotency- and cardiomyocyte-specific markers, we have concluded that progeroid hiPSC-CMs appear to reach a more advanced level of maturation compared to healthy control cells. Additionally, we have performed proteomics analyses of healthy hiPSC-derived cardiomyocytes overexpressing circZNF609. The results have allowed us to offer promising insights on the potential mechanisms by which circRNA-ZNF609 functions in the heart. First, we have observed that a significant number of the differentially expressed proteins are involved in mitochondrial homeostasis. Then, when focusing on the interactome of the peptides encoded from circZNF609, we have detected that several of the enriched proteins are associated with RNA-dependent processes, as translation initiation, nuclear and mitochondrial splicing and ribosome and spliceosome assembly, as well as cell cycle regulation. Collectively, these findings point to the active

involvement of circZNF609 in the aberrant regulation of these processes in human cardiomyocytes from patients afflicted by this specific form of laminopathy.