Platelets and platelet extracellular vesicles as messengers in vascular inflammation

Valorisation

The valorisation of research, i.e. the impact and contribution of research to society has increasingly gained importance. According to the National Valorization Committee 2011:8, “Knowledge valorization refers to the process of creating value from knowledge, by making knowledge suitable and/or available for social (and/or economic) use and by making knowledge suitable for translation into competitive products, services, processes and new commercial activities." The work presented in this thesis aimed to investigate fundamental basic research such as cellular communication in a clinical relevant context of cardiovascular diseases. Cardiovascular diseases (CVD) affected an estimated 17.7 million people in 2015 and represent 31% of all global death (WHO, May 2017). The underlying cause for CVD is atherosclerosis, a disease that is characterized by a slowly progressing lesion formation of the arterial wall, mediated by oxidized lipoprotein-triggered immune cell influx, accumulation of dead cells and foam cells, and vascular cells as well as the reorganization of smooth muscle cells and the extracellular matrix. These slowly growing, stable plaques expand gradually and are covered by a fibrous cap and are not prone to rupture. In contrast, unstable plaques that build up more rapidly as a result of increased lipid deposition have thin fibrous caps that are more vulnerable. These unstable plaques might rupture, initiate platelet activation and coagulation. Subsequently, this might lead to occlusion of the vessel and to severe cardiovascular outcomes such as myocardial infarction (MI) or stroke [1, 2]. Effective treatment of acute MI by reperfusion therapy promotes the restoration of blood flow to the ischemic myocardium. The ensuing inflammatory reactions, as a response to tissue injury continue to damage the heart tissue during reperfusion, which may lead to further irreversible cardiomyocyte death. Eventually, reparative mechanisms become predominant and replace the injured area with scar tissue, that does not actively contract [3, 4]. Despite the discovery of the cardioprotective effects of the innate adaptive responses evoked by different conditioning strategies (pre-, post- and remote conditioning) and intensive research to identify key cellular mechanisms and drug targets [5], the currently available treatment options for myocardial ischemia/reperfusion (I/R) injury are still suboptimal to suppress the wave of inflammation and to

decrease subsequent scar size [3]. Therefore, new therapeutic, cardioprotective strategies are urgently needed to control the acute inflammatory reaction after myocardial I/R injury. One of our aims within this thesis was to evaluate a peptide (MKEY), that has been specifically designed to block the interaction of the platelet-derived chemokines CCL5 and CXCL4 in a mouse model of myocardial ischemia/reperfusion. These chemokines potently trigger the recruitment of leukocytes to the sites of inflammation, particularly upon heteromerization [6–8]. Inhibition of the heteromerization of CCL5 and CXCL4 by MKEY during myocardial I/R reduced infarction size and preserved heart function. MKEY treatment significantly reduced the influx of inflammatory cells following myocardial I/R, as revealed by specific staining for neutrophils and monocytes/macrophages. Moreover, MKEY treatment led to a significant reduction of citrullinated histone 3 in the infarcted tissue, indicating that MKEY can prevent the formation of neutrophil extracellular traps (NETs) in vivo. The peptide MKEY might represent a new therapeutic approach for the treatment of atherosclerosis and myocardial infarction. This strategy potentially has a clinical impact, having advantage over direct antagonism of chemokines or their receptors, by preserving the therapeutic effect of specifically blocking chemokines, yet also reducing the side effects and maintaining normal immune defence. Platelets are important contributors in in-stent thrombosis and restenosis [9]. Antiplatelet therapy using aspirin and clopidogrel has been the standard of care for prevention of thrombosis following a coronary stent implantation [10]. Since these anti-platelet medications target platelet activation and aggregation, they have been associated with increased bleeding risk. Precise understanding and knowledge of the underlying mechanisms to identify key mediators is required to develop more effective therapeutics. Within this thesis we have investigated the role Junctional Adhesion Molecule A (JAM-A) on platelets. Previous studies have already investigated the roles of endothelial- and leukocyte JAM-A in atherosclerosis. On leukocytes, JAM-A has protective effects in atherosclerotic lesion formation, whereas endothelial JAM-A promotes plaque formation [11]. In this study, platelet-specific deletion of JAM-A led to increased neointima formation after vascular injury through the increased recruitment of inflammatory cells and increased SMC proliferation. These findings further emphasize the cell-specific role of JAM-A and might be clinically relevant for the development of more effective anti-platelet medication. Another important aspect is the early identification of patients at high cardiovascular risk via new specific biomarkers. As the research field of extracellular vesicles (EV) is continuously expanding and the experimental detection methods simultaneously

evolve, EV might represent potential new biomarkers. The standardization of the isolation and detection of EV from biologic specimen for a clinical setting is therefore urgently needed. Within this thesis we have reviewed current isolation, quantification, and characterization methods. Concluded was that processing of EV from biologic specimen is still challenging due to their size and heterogeneity. Moreover, EV might represent a new complex mechanism of cellular communication during health and disease. The role of platelet EV released from ageing platelets on the functional modulation of smooth muscle cells (SMC) in the context of vascular remodeling has been investigated. We have found that platelet EV induced a pro-inflammatory SMC phenotype, characterized by the increased SMC proliferation, migration, cytokine release and recruitment of monocytic cells. Particularly patients who receive platelet concentrates that accumulate EV might potentially be at risk of a pro-inflammatory response. Moreover, particularly during cardiovascular disease, circulating platelet EV might predict the onset of underlying vascular inflammatory processes. For the development of new biomarkers, fundamental research is necessary to realize the standardization of processing EV from biologic specimen and to understand molecular mechanisms of (platelet) EV during health and disease.

References

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