Changes in protein citrullination profile and chromatin structure induced by peptidyl-arginine deiminases activity and their consequences for the hemostatic functions of endothelial cells

Hemostasis is the interplay between multiple processes, consisting of cellular and biochemical mechanisms that maintain blood fluidity and prevent blood leakage from damaged vessels. The human hemostatic system consists of four main components: the coagulation cascade and fibrinolytic system, as well as cellular components such as platelets and vascular endothelium. The coagulation cascade results in the formation of fibrin clots that stop bleeding from the damaged vessels. Fibrinolysis is a series of events responsible for the dissolution of clots when damage is repaired. Blood coagulation disorders are a group of highly prevalent hematological disorders, the most known such as hemophilia (types A and B) or von Willebrand disease. They are almost always inherited, but in rare cases, they can also develop later in life, and increasing evidence points to some non-hereditary factors that may cause or increase the severity of blood coagulation disorders. Epigenetics – the regulation of gene expression by mechanisms that do not induce changes in the DNA sequence, such as DNA methylation and histone protein modifications, plays a key role in maintaining the proper functioning of the whole organism. The epigenetic regulation of hemostasis is attracting increasing interest, but findings are still scarce, and very little is known about the potential role of histone post-translational modifications (HPTMs) in regulating the expression of coagulation and fibrinolytic factors in endothelial cells (ECs).

Vascular endothelial cells line the inner walls of blood vessels, which makes them key players in regulating blood flow, coagulation, and fibrinolysis. The importance of ECs function for the whole human organism has been widely studied, for instance, in angiogenesis and inflammatory response. In blood vessels, ECs act not only as a barrier between the circulating blood and extravascular environment but also produce and secrete countless molecular agents that maintain hemostasis, such as von Willebrand Factor (vWF), tissue factor (TF), and tissue plasminogen activator (t-PA). The strategic location of ECs on the walls of blood vessels makes them highly susceptible to epigenetic modulation of their function. For instance, via the action of dietary components, such as polyphenols from fruits and vegetables. Surprisingly, the role of epigenetic regulation of gene expression, including that of HPTMs, in the context of hemostasis seems to be understudied.

Protein citrullination is a relatively novel and poorly understood protein modification that can also be introduced into histones. The reaction is based on the change of the protein-bound arginine to citrulline, which may alter the overall charge of the protein and result in loss of its function. In the case of histones, citrullination was shown to increase the expression of some genes due to loosening of the chromatin structure. The reaction is performed by a family of enzymes called peptidyl-arginine-deiminases (PADs), and increased levels of these enzymes and by extension increased citrullination are associated with the pathology of several autoimmune disorders, most notably rheumatoid arthritis. Recently, the role of these enzymes has been studied in the context of hemostasis, but as this field of research is developing, there is still much that awaits exploration.

Our group recently started researching the impact of protein citrullination on the flagship function of ECs – angiogenesis, the formation of new blood vessels from pre-existing ones which is important during development, the healing of wound but also cancer progression. We treated ECs with pharmacological inhibitors of PADs enzymes and found that this resulted in a significant alteration of the angiogenic potential of these cells by lowering the expression and secretion of important angiogenic regulators. Surprisingly, our research brought exciting results about the levels of a few selected hemostasis-related factors, such as TF and SERPINs, which we originally researched for their role in angiogenesis and not blood coagulation.

This Preludium project is based on these unexpected findings and aims to further analyze the potential role of protein citrullination in regulating hemostasis from an endothelial cell perspective, and its role during blood coagulation and fibrinolysis. To that end, we will establish two endothelial cell models *in vitro*: endothelial colony-forming cells (ECFC) isolated from blood and human umbilical vein endothelial cells (HUVEC) isolated from the veins of umbilical cords. We will then treat these ECs with PAD inhibitors to stop the process of protein citrullination and analyze the effects this has on the functioning of ECs as a barrier in the vessel wall and inspect the expression, secretion, and activity of selected hemostasis-related factors. The discovered links between histone citrullination and the expression profile of these factors, we will analyze in depth using next generation sequencing (NGS).

The expected results will improve our knowledge of the role of protein citrullination and, most notably, histone citrullination in regulating the hemostatic role of endothelial cells. Identification of epigenetic regulation of hemostasis *via* histone citrullination could lead to the development of new targets for the treatment of bleeding disorders and potentially open a new area of endothelial cell epigenome research.