Genetic mechanisms of inherited bleeding disorders as a basis for personalised medicine approaches

CHAPTER 8

Impact

The human body physiologically possesses an innate ability to prevent excessive bleeding and thrombotic episodes. These two mechanisms are constantly balancing each other in haemostasis. Mutations in genes involved in haemostasis may perturb this delicate equilibrium, thereby predisposing to bleeding or thrombosis.

This thesis focusses on genetic variants responsible for inherited bleeding disorders with the aim of designing personalised therapies to specifically target the cause of the disorder.

For FV deficiency there is currently no replacement therapy available. The treatment and prophylaxis of these patients relies on plasma transfusion. This treatment requires hospitalisation and, despite the extraordinary advances in transfusion medicine, might expose patients to risks such as allergic reactions and overload of the cardiovascular system. Therefore, we investigated in a pre-clinical setting known and new molecules as possible therapeutics for FV deficiency caused by a specific nonsense mutation. We demonstrated that this nonsense mutation has favourable characteristics for correction. This approach has the potential to be extended to other nonsense mutations with the same favourable characteristics. One of these molecules (PTC-124), which has an oral route of administration, has been approved for the treatment of Duchenne muscular dystrophy due to nonsense mutations. A potential approval for FV-deficient patients would alleviate the burden of repeated transfusion and ameliorate their quality of life. In this thesis we also investigated molecular approaches to modify the expression of FV-short, a low-abundance isoform of FV. The overexpression of FV-short is responsible for increased levels of an important anticoagulant protein (TFPI), causing the bleeding diathesis. To this date, 4 families with genetic mutations that cause FV-short overexpression, high TFPI levels and bleeding tendency have been reported. In an attempt to develop a treatment for these disorders, we tried to modulate the expression of FV-short using molecules that target pre-mRNA splicing (morpholino antisense oligonucleotides) or mRNA stability and translation (siRNA). We observed a dose-dependent decrease of FV-short upon treatment with antisense oligonucleotides. Since our findings were observed *in vitro*, more studies are needed to assess safety and efficacy for a possible clinical use. In addition, this molecular approach could be extended to other bleeding disorders where TFPI is the determinant of bleeding severity.

Although our results are still far from a clinical use, these findings contribute to understanding the potential of a molecular therapy and may stimulate further studies investigating this approach in more complex systems in view of a possible future application in patients.

The findings of this thesis have been presented several times at national and international conferences, where they raised interest and stimulated the scientific discussion with other researchers and exchange of ideas and perspectives.

Part of my studies has been carried out using blood of real patients. One of these patients was very enthusiastic about and interested in the research we were conducting on his rare disease. His appreciation and expectations made me realise the societal impact of our work.