Coagulation factur V deficiency

Valorisation

Science was originally a curiosity-driven activity with no other purpose than to understand and explain natural phenomena. Only later it was realized that the knowledge gained by exploring nature could be employed to solve practical problems. This dichotomy is still reflected in the current distinction between basic and applied research.

My PhD project was dedicated to the study of the genetics and biochemistry of factor V (FV) deficiency, a rare inherited bleeding disorder for which no optimal treatment is available yet. Most of the work described in this thesis can be regarded as basic research and was done with the primary aim of gaining more insight into the molecular bases of this genetic disease. However, some of my results may find practical application in the near or distant future.

First of all, I have developed and validated a multiplex ligation-dependent probe amplification (MLPA) assay for the detection of large deletions and duplications in the *F5* gene. This assay can be performed in any molecular biology laboratory and may be implemented immediately in the molecular diagnostics of FV deficiency. The company that developed the MLPA technique expressed interest in my assay and invited me for a seminar.

In addition, I have shown in both *in vitro* and *ex vivo* models that some *F5* splicing mutations responsible for (severe) FV deficiency are amenable to antisense-based RNA therapy using antisense oligonucleotides or engineered U7 small nuclear RNA (U7snRNA). Although these studies are promising, the *in vivo* translation of this form of therapy is still hampered by the lack of suitable animal models and by the rare occurrence and genetic heterogeneity of FV deficiency, each mutation requiring a different custom-made oligonucleotide (or U7snRNA) with its own pharmacological/toxicological profile.

Finally, I have provided additional evidence that the level of plasma full-length tissue factor pathway inhibitor (TFPI) is an important determinant of the bleeding tendency in FV-deficient patients. This finding suggests that TFPI might be a suitable target for

pharmacological interventions aimed at preventing life-threatening bleeding in severe FV deficiency. In this respect, FV-deficient patients may benefit from the TFPI inhibitors that are already being developed and tested for the treatment of haemophilia, a much more common bleeding disorder.

Since FV deficiency is a rare disease, with an estimated incidence of 1 in 1 million in the general population, it is unfortunately not an attractive target for the pharmaceutical industry. This is regrettable, as some FV-deficient patients bleed as much and as severely as haemophiliacs, but enjoy a far lower healthcare standard, even in the Western world. Despite remarkable advances in transfusion medicine, the repeated administration of fresh frozen plasma exposes FV-deficient patients to the risk of transfusion reactions, allergic reactions and the transmission of infectious agents, besides taking a toll on the patients' cardiovascular systems on the longer run. On the other hand, the therapeutic development of antisense molecules targeting individual mutations for the personalised treatment of a single patient or only a few patients is hardly feasible, unless the regulations for clinical testing and licensing are specifically modified, as currently being discussed for other rare and/or life-threatening genetic diseases with unmet medical needs (Aartsma-Rus *et al*. Translational and regulatory challenges for exon skipping therapies. *Hum Gene Ther* 2014; 25: 885-892). In this context, pharmacological inhibition of TFPI by means of antibodies, peptides, aptamers or small molecules could represent a valid therapeutic alternative, suitable for all FV-deficient patients irrespective of their mutation(s).

In conclusion, given the complications associated with the current treatment of FV deficiency, it is highly desirable that the long-awaited FV concentrate (currently undergoing pre-clinical testing) and/or other forms of therapy, such as those outlined in this thesis, will become available soon.