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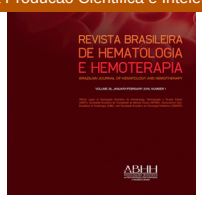

Scientific Comment

Bioengineering coagulation factors for improved hemophilia treatments
Comment on: the mutation F309S increases FVIII secretion in human cell line☆

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The implementation of prophylaxis with regular factor VIII (FVIII) replacement for patients with hemophilia radically changed the clinical presentation of this condition, from a disease characterized by progressive disabling musculoskeletal complications, to one compatible with an active and virtually normal lifestyle. In Brazil, although most adults with severe hemophilia still suffer the impact of musculoskeletal complications in their quality of life,[1] the recent implementation of universal prophylaxis will certainly change this situation for the next generation of patients. This scenario posits a challenge to government and healthcare providers, for whom guaranteeing a reliable and safe source of FVIII concentrate has become more than ever, a strategic issue. This is the context that underscores the importance of the study by Fantacini et al. in this issue of the Revista Brasileira de Hematologia e Hemoterapia, in which the authors used a combined strategy to improve recombinant FVIII production, based on 'rational mutagenesis', chemical chaperones, and human cell lines.[2]

Rational mutagenesis consists in the introduction of DNA variations (and hence, amino acid changes) aimed at improving the function of a recombinant protein, based on previous knowledge about protein structure and function. The power of rational mutagenesis was well-illustrated by the identification in 1988 of a residue in coagulation factor IX (FIX) whose change increased FIX function.[3] This occurred 11 years before the report of an analogous change in this very residue in a family with extremely high levels of FIX activity.[4] In this report, the authors used a FVIII variant with a point mutation (FVIII F309S) in a region that determines the magnitude of ATP-dependence of FVIII secretion. This mutation was rationally designed in 1997 by Swaroop et al., based on a previous demonstration that this specific region of FVIII could bind a protein that limited its secretion.[5] By changing a single amino acid in this region, based on the structure of factor V, the authors were able to decrease the ATP-dependence of the secretion process, thereby enhancing production by 2.3-fold, with no apparent impact on protein function. Fantacini et al. went further, by introducing this mutation in a human cell line (HEK), and coupling this strategy with the use of chemical chaperones to further improve FVIII secretion. Although the use of chaperones only improved wild type FVIII secretion (possibly because little or no FVIII F309S was left to be secreted), the authors were able to reproduce the results of Swaroop et al. in a human cell line, and to demonstrate that recombinant FVIII F309S maintained normal function as measured both *in vitro* and *in vivo*, in a tail clip assay in hemophilia A mice.[2]

One of the major issues of site-specific mutagenesis in protein engineering is its still widely unpredictable

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immunological consequences. In fact, the extreme sensitivity of the immune system to even minor changes was demonstrated by the development of inhibitory antibodies in patients exposed to a factor VIIa variant (vatreptacog alpha), characterized by a 3-amino acid change that increased its activity, but turned it into a immunogenic molecule.[6] The fact that this outcome was not anticipated in animal models highlights the importance of very careful pre-clinical and clinical development programs for bioengineered proteins.

Another important aspect of the report of Fantacini et al. is that, as opposed to the vast majority of currently available recombinant FVIII products (expressed in rodent cell lines), the authors use a human cell line in their expression system. Besides limiting the exposure to animal proteins, the use of human cell lines can reduce differences in post-translational modifications in the expressed protein. This could be particularly interesting in this field, since the development of inhibitory antibodies to FVIII is currently the main challenge of hemophilia treatment. Although the clinical relevance of this strategy to inhibitor development is yet to be proved, the recent introduction of the first commercial recombinant FVIII product expressed in a human cell line[7] will allow this hypothesis to be tested.

In conclusion, the study of Fantacini et al. represents an additional step of a Brazilian group toward mastering the technology of recombinant coagulation factor concentrate production,[8] which as previously stated, should be regarded as a strategic area of research.

Conflict of interest

The authors declare no conflicts of interest.

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