2.07 Novel therapeutic products and approaches (including gene therapy)

IDENTIFICATION OF NOVEL HYPERACTIVE RFVIIA BYPASSING MOLECULES INFERRED BY ANCESTRAL SEQUENCE RECONSTRUCTION APPROACH

M.F. Testa, I. Passerini, P. Santos, A. Benazzo, S. Ghirotto, F. Bernardi, A. Branchini, M. Pinotti, D. Balestra

Dep. of LSB, University of Ferrara, Italy

Background and Aims. Recombinant activated factor VII (rFVIIa) is the most common by-passing agents (BPA) licensed for the treatment of HA and HB patients with inhibitors, and it has proven to be safe and efficacious, but its dosing remains problematic. In this view, a hyperactive and/or longer acting rFVIIa variant is highly desirable. Here, we propose novel rFVIIa variants with improved specific activity.

Materials and Methods. We collected FVII homologues of a broad range of species from sequence databases (n=5000), used to perform multiple-sequence alignments. Through the GRASP tool, we inferred a phylogenetic tree considering all species, namely Ancestral Sequence Reconstruction (ASR) approach, mapping the evolutionary relationships among FVII homologs. F7 sequences were restricted to species (n=61) whose evolutionary context suggests potential favored coagulation system. Selected rFVIIa sequences (n=20) were transiently expressed in HEK293 lines, and protein levels analyzed by ELISA and PT/FXa generation assays.

Results. The rFVIIa variants were selected (n=20) from phylogenetic trees composed by 61 contemporary F7 sequences and 59 ASR sequences based on i) structural features (n=12) and ii) ASR-derived sequences (n=8), considering basal and divergency nodes among species. Among expression data of contemporary rFVIIa variants, we identified four out of 12 (var3 FVII:C 148% of WT, var6 FVII:C 66%, var9 FVII:C 360% and var10 FVII:C 110%) showing remarkable higher coagulant activity (3-fold) in PT assays. Evaluation of antigen levels of novel variants (var3 FVII:Ag 123% of WT, var6 FVII:Ag 36%, var9 FVII:Ag 300%, var10 FVII:Ag 59%) clearly demonstrated significantly higher (var3 Sp.Ac:2.0; var6 3.1; var9 2.0;var10 3.2) specific activity compared to rFVIIa WT (1.6). These data were further confirmed by the highly sensitive FXa generation assay resulting in comparable fold (ratio Sp.Ac. variants/Sp.Ac. rFVIIa WT) with PT. Data obtained from expression of ASR variants showed 5 out of 8 nodes with marked coagulant hyperactivity (N10, N11, N18, N28; N30) and significantly higher specific activity compared to rFVIIa WT. Interestingly, we found out that the F7 sequences that one exist in the past with hyperactive features ensue from a basal node between carnivores and cetaceans or primates. In-silico immunogenicity studies are ongoing.

Conclusions. Our data, through the innovative ASR approach, revealed hidden FVII variants with improved features that once existed in the past and promises to redefine the landscape of hemophilia treatment. Importantly, the development of novel rFVIIa variants will enable the exploration of new molecular insights, considering the FVII involvement in several hemostatic pathways.