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Utilizing Platelets as a Targeted for Gene Therapy of Hemophilia a and Hemophilia B

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Hemophilia A is a genetic bleeding disorder resulting from a deficiency of coagulation protein factor VIII (FVIII). FVIII Protein replacement therapy is effective, but up to 30% of patients will develop inhibitory antibodies (inhibitors) against FVIII, rendering protein replacement treatment useless. Gene therapy is an attractive alternative for the treatment of hemophilia A as it may provide a cure if successful. We have developed a gene therapy protocol in which FVIII expression is ectopically targeted to platelets under control of the platelet-specific αIIb promoter, termed 2bF8. Using a transgenic mouse model, we have demonstrated that FVIII is stored together with its carrier protein von Willebrand factor (VWF) in platelet α-granules as determined by electronic microscopy and confocal microscopy. Our studies have demonstrated that platelet-derived FVIII can rescue the bleeding phenotype in hemophilia A mice even in the presence of high-titers of inhibitors. Our further studies demonstrate that 2bF8 lentiviral gene delivery to hematopoietic stem cells (HSCs) by HSC transduction followed by syngeneic transplantation can not only effectively restore the hemostasis but also induce antigen-specific immune tolerance in hemophilia A mice. When a similar protocol is applied to hemophilia B, which is a genetic bleeding disorder resulting from a deficiency of factor FIX (FIX), we show that FIX can also be stored in platelet α-granules when it is driven by the platelet-specific αIIb promoter (2bF9) and that platelet-derived FIX can rescue the bleeding phenotype in hemophilia B mice. Unlike platelet-FVIII in hemophilia A mice, the clinical efficacy of platelet-derived FIX is limited in hemophilia B mice in the presence of anti-FIX inhibitors. This could be because FIX does not have a carrier protein to protect it, so inhibitors can freely inactivate functional FIX once it is released from platelets. However, using hyperfunctional 2bF9 gene therapy can eradicate inhibitors and ultimately provide therapeutic protein once inhibitors drop to undetectable. Together, our studies demonstrate that platelet-targeted gene therapy is a promising approach for the treatment of hemophilia A and hemophilia B.

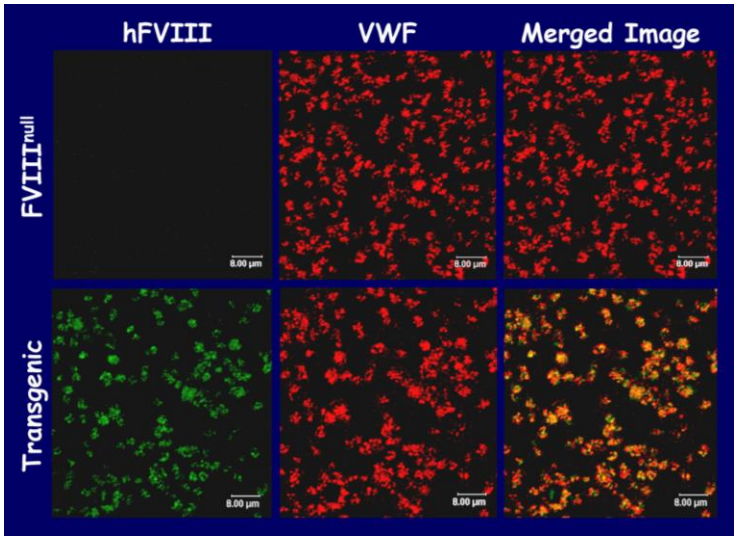

Figure 1. Immunofluorescent confocal microscopy shows FVIII and VWF colocalization in platelets of 2bF8 transgenic mice [Shi et al. J Clin Invest 2006, 116(7):1974-82].

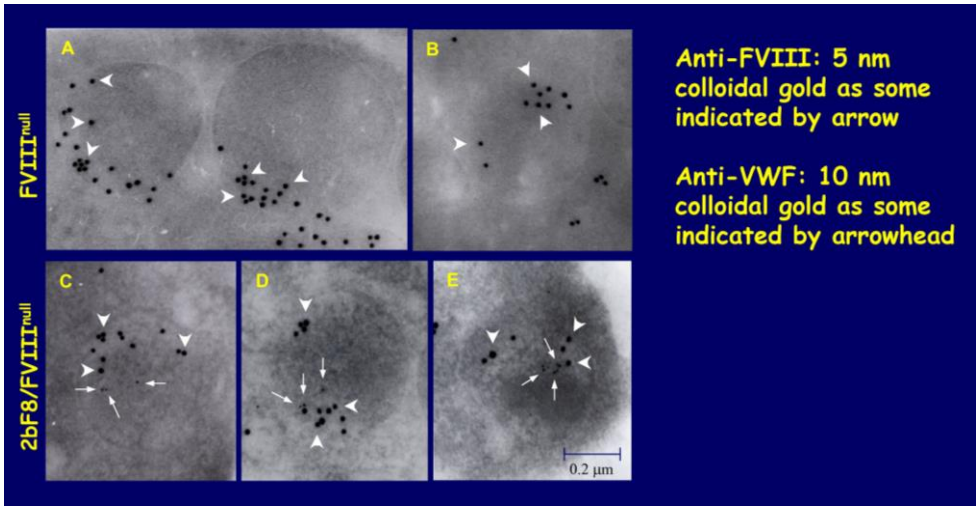

Figure 2. Immunogold electron microscopy confirmed FVIII colocalized with VWF in a-granules in the platelets of 2bF8 transgenic mice [Shi et al. J Clin Invest 2006, 116(7):1974-82].

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