Long-term correction of VWD via Sleeping Beauty transposon-mediated gene therapy

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Von Willebrand disease

What?
Most common inherited bleeding disorder
Caused by quantitative or qualitative defects in VWF
Most severe form: type 3 VWD (total absence of VWF)

Treatment?
Replacement of defective protein: DDAVP or VWF concentrates (or rVWF)
Replacement of defective gene: gene therapy
Gene therapy for VWD: failures and successes

- Chimeraplasty: disaster
  (De Meyer et al., Thromb Res, 2007)

- Lentiviral vectors: success but not with ex vivo approach
  (De Meyer et al., Blood, 2006)

- Non viral targeting of liver: high and functional VWF but transient
  - High VWF expression (> 1000%) of good quality
  - Correction of bleeding phenotype
  - But short-lasting effect (2-3 weeks)
  (De Meyer et al., ATVB, 2008)

Can we target the liver for long-term transgene VWF expression using the non-viral Sleeping Beauty transposon technology?
Sleeping Beauty transposon technology

**Experimental design:**

VWF transposon plasmid + SB100x transposase plasmid

hydrodynamic gene delivery in VWF KO mice

VWF gene integration? VWF expression? Bleeding correction? ...
VWF expression after SB-mediated gene therapy

the CAG promoter

The good news: sustained transgene VWF expression for > 1 year

The bad news: expression levels are very low
VWF expression after SB-mediated gene therapy

the CAG promoter

Splinkerette PCR: analysis of transposon integration sites

mouse 1: sacrificed d42
Chromosome 19, cytochrome P450
Chromosome 11, cytochrome tensin-4 precursor

Mouse 2: sacrificed d126
Chromosome X, TGF-B induced factor homebox-2

Mouse 3: sacrificed d322
Chromosome 5, TBC-1 domain family member 1

VWF cDNA is integrated in VWF KO mouse liver genome
VWF expression after SB-mediated gene therapy
the liver-specific AAT promoter

Liver-specific promoter greatly enhances long-term VWF expression

Males (n=6) approx. 35%
Females (n=6) approx. 8%
Bleeding time

Measurement of tail clipping bleeding time 3 months after gene transfer

Mice with highest expression levels are corrected
Conclusions

- Large VWF cDNA (8.4 kb) can be transposed by Sleeping Beauty transposon technology
- VWF cDNA can be inserted in liver genome
- Long-term VWF expression can be obtained
- Correction of bleeding phenotype can be established
  
  proof of concept for long-term gene therapy for VWD

Current and future strategies:

sandwich-transposon  Extra efficient SB100x  Further optimized transposon system

Long-term assessment of phenotypic correction (also in thrombosis models)
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