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Human artificial chromosomes for gene delivery

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Successful examples using two types of HAC

I. Stable HAC

1. 21HAC (Fig1, 2)
   1) DYS-HAC for Duchenne muscular dystrophy (DMD) therapy(Fig3)
   2) FVIII-HAC for hemophilia-type A therapy(Fig4)
   3) Yamanaka's factor-HAC for iPS generation(Next poster #1586)
   4) CYP3A-HAC for humanized P450model mouse for drug screening(Fig5)

2. MI-HAC (21HAC derivative)

Multiple gene insertion into HAC(Fig6)

Summary

Gene therapy has been envisioned to provide a direct and permanent correction of genetic defects. Although a number of different approaches have been attempted to achieve efficient gene transfer and long-term gene expression, this challenging task remains unfulfilled as all current methods have some limitations. For example, random integration of conventional gene delivery vectors.

An alternative solution to these problems could be the use of human artificial chromosome (HAC) vectors. Our HACs are exogenous mini-chromosomes artificially created from a normal human chromosome 21. The HACs can be transferred into host cells, mainly by microcell-mediated chromosome transfer (MMCT). The HACs can faithfully mimic the normal pattern of gene expression because they can hold complete genomic loci, including the upstream and downstream regulatory elements. It might also be possible to maintain the long-term correction of defective genes, because these vectors are mitotically stable throughout many cell divisions, at least in human cells.

Several experimental data showed that the HACs would be advantageous over current gene delivery methods in several points. Thus, a combination of stem cell-based tissue engineering with HAC technology may open up new avenues for gene and cell therapies, and for the developments of transgenic animals.

Fig.1 Characteristics of our human artificial chromosome (HAC) vector

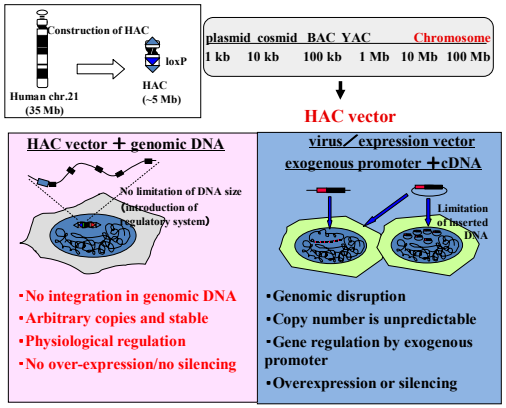


Fig.2 Gene cloning system on the HAC vector

HAC can be transferred to other cells by microcell-mediated chromosome transfer (MMCT).

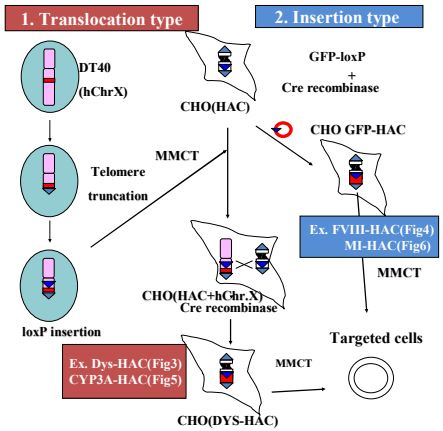
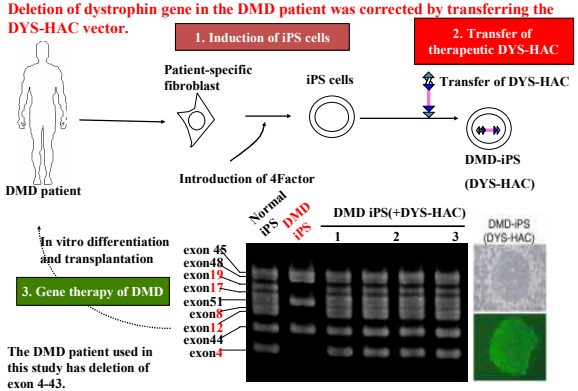


Fig.3 Schematic diagram of gene and iPS-based cell therapy using HAC

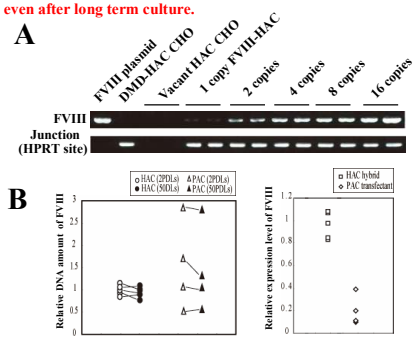
Deletion of dystrophin gene in the DMD patient was corrected by transferring the DYS-HAC vector.



Exons of the red line were deleted in DMD-patient derived iPS cells.

Fig.4 Trangene stability and sustained transgene regulation associated with FVIII-HAC

FVIII on the HAC was expressed in copy-number dependent manner. FVIII in hiMSCs(FVIII-HAC) was stably expressed even after long term culture.



(A) Representative data from PCR analysis of genomic DNA confirm the structure of the reconstituted HPRT junction and the FVIII transgenes. (B) Graphic representation of the FVIII transgene and its expression in hiMSC hybrids and transfectants after 2 (initial stage of cloning) and 50 PDLs (long-term culture after selection withdrawal) . Genomic PCR analysis of FVIII and the β-Actin control is shown in the left graph. RT-PCR analysis of relative FVIII expression at 50 PDLs to 2 PDLs is shown in the right graph.

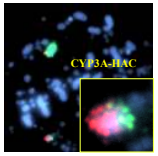
Fig.5 HAC utility for animal transgenesis

CYP3A genes cloned into the HAC were expressed in tissue specific and developmental stage-specific manner.

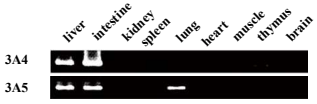
-Genomic size of human CYP3A cluster is ~1Mb.

-The CYP3A family is the most important drug metabolizing enzyme.

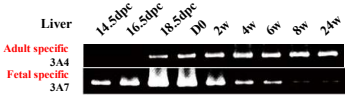
-The CYP has species specific function and inducibility.



HAC:red
CYP3A cluster: green



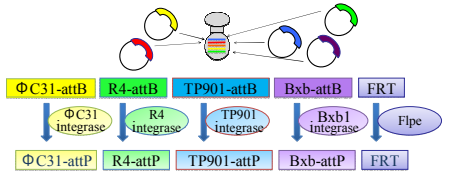
CYP3A4 and CYP3A5 were expressed mainly in liver and intestine.



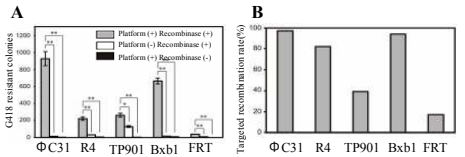
Adult specific CYP3A4 were expressed in adult stage and fetal specific CYP3A7 were expressed in fetal stage.

Fig.6 Multiple gene insertion into HAC

The system enables irreversible integration of multiple genes (plasmid , BAC or PAC)at the specific locations in HAC. The integration efficiency is very high (>95%) in the case of ΦC31(B).



A direct comparison of the recombination efficiency mediated by phage integrases and FLPe.



(A) In vitro integration efficiency after integrases and FLPe-mediated integration. A summary of the counted colonies for each condition after crystal violet staining is shown. Data were corrected for average colony numbers in one of the ten dishes and expressed as means 6 SE. Student's t-test was used to determine statistically significant differences. Differences at P,0.05 were considered significant. *P,0.05, **P,0.01. (B) Site-specific recombination efficiency was determined by genomic PCR.

References

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6. Human artificial chromosome with a conditional centromere for gene delivery and gene expression. Iida Y et al, DNA Res 2010.

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