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Site-specific Cellular Modification by Cre-incorporating Integrase-defective Retroviral Vectors

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Retroviral vectors have been employed in various biotechnology fields such as gene therapy [1], generation of transgenic animals [2] and induced pluripotent stem cells (iPSCs) [3]. However, the nearly random integration property may cause problems such as insertional mutagenesis and gene silencing. Therefore, the development of targeted transgene integration system is desired for modification of retroviral vectors. We have achieved retroviral insertion of transgene into a pre-determined site of chromosome in CHO cells using integrase-defective retroviral vectors (IDRVs) and Cre-recombinase mediated cassette exchange (RMCE) [4]. In this case, founder cells were transfected with a Cre expression plasmid prior to retroviral transduction. To apply site-specific gene modification for hard-to-transfected cells, it is desired to encapsulate both gene of interest and Cre protein into the retroviral virion. In this study, we attempted to generate novel hybrid IDRVs, which can deliver both viral genome and enzymatically active Cre.

First, we designed a fusion protein-expression vector (pGPM-Cre), in which a Cre gene was inserted into the gag-pol precursor genes (pGPM). Cre-incorporating IDRV particles (Cre-IDRV) were produced by 293FT cells co-transfected using IDRV producing plasmids (pQMSCV/NE, pGPM and pVSV-G) and a pGPM-Cre plasmid. Western blot analysis revealed that Cre was incorporated into retroviral virions. A Cre-IDRV encoding marker genes (*ATG-deleted-Neo*^{r}*/IRES/EGFP*) flanked by wild-type and mutant loxP sites was infected into founder CHO cells (CHO/F17), in which a single copy of an expression unit containing a CMV promoter, an ATG codon and marker genes (*ATG-deleted-Hyg*^{r}*/IRES/DsRed*) flanked by corresponding *loxP* sites was introduced into the genome. We observed appearance of G418-resistant colonies expressing GFP. Moreover, site-specific integration of transgene was confirmed by PCR and sequence analyses. These results indicate that bioactive Cre protein was incorporated into virions and site-specific recombination between retroviral DNA derived from IDRV and cellular genome was mediated by Cre-RMCE. The Cre-IDRV system may expand the applicability of retroviral vectors.

[1] Qaism *et al*., Progress and prospects: Gene therapy for inherited immunodeficiencies. *Gene Therapy,* 16:1285-1291, 2009.
[2] Kamihira M *et al*., Production of chimeric monoclonal antibodies by genetically manipulated chickens. *Journal of Biotechnology*, 141:18-25, 2009.
[3] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126:663-676, 2006.
[4] Huang S *et al*., Cre recombinase-mediated site-specific modification of a cellular genome using an integrase-defective retroviral vector. *Biotechnology and Bioengineering*,107:717-729, 2010.