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Identification and characterization of MYH9 locus for high efficient gene knock-in and stable expression in mouse embryonic stem cells

Aibing Wang
Hunan Agricultural University, China

Targeted integration of exogenous genes into so-called safe harbors/friend sites, offers the advantages of expressing normal levels of target genes and preventing potentially adverse effects on endogenous genes. However, the ideal genomic loci for this purpose remain limited. Additionally, due to the inherent and unresolved issues with the current genome editing tools, traditional embryonic stem (ES) cell-based targeted transgenesis technology is still preferred in practical applications. Here, we report that a high and repeatable homologous recombination (HR) frequency (>95%) is achieved when an approximate 6kb DNA sequence flanking the MYH9 gene exon 2 site is used to create the homology arms for the knockout/knock-in of diverse nonmuscle myosin II (NM II) isoforms in mouse ES cells. The easily obtained ES clones greatly facilitated the generation of multiple NM II genetic replacement mouse models, as characterized previously. Further investigation demonstrated that though the targeted integration site for exogenous genes is shifted to MYH9 intron 2 (about 500bp downstream exon 2), the high HR efficiency and the endogenous MYH9 gene integrity are not only preserved, but the expected expression of the inserted gene(s) is observed in a pre-designed set of experiments conducted in mouse ES cells. Importantly, we confirmed that the expression and normal function of the endogenous MYH9 gene is not affected by the insertion of the exogenous gene in these cases. Therefore, these findings suggest that like the commonly used ROSA26 site, the MYH9 gene locus may be considered a new safe harbor for high-efficiency targeted transgenesis and for biomedical applications.

Biography

Aibing Wang obtained BS and MS from Hunan Agricultural University (HUNAU) in 1998 and 2001, respectively; PhD majored in Biochemistry and Molecular Biology from Peking Union Medical College (PUMC) in 2005. He was a Visting and Research Fellow in National Heart, Lung and Blood Institute (NHLBI) of National Institutes of Health (NIH) during 2005-2011. He worked as a Biologist and Senior Biologist in NIH during 2011-2016; He joined in CVpath Insitute Inc as a Senior Scientist in 2016; Currently, he is a Professor of Molecular biology and virology in the School of Veterinary Medicine of Hunan Agricultural Univesity since 2016. He has obtained 3 awards from NIH and 3 grants from the Chinese government, applied for 3 patents and published 45 peer-reviewed publications. His current research interests mainly include: investigating the gene functions or molecular mechanisms associated with virus infection and other diseases; exploring the development of virus detection and vaccine using cutting-edge technologies such as CRISPR/Cas9.

bingaiwang@hunau