REVIEW ARTICLE

Combinations of chromosome transfer and genome editing for the development of cell/animal models of human disease and humanized animal models

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Abstract
Chromosome transfer technology, including chromosome modification, enables the introduction of Mb-sized or multiple genes to desired cells or animals. This technology has allowed innovative developments to be made for models of human disease and humanized animals, including Down syndrome model mice and humanized transchromosomic (Tc) immunoglobulin mice. Genome editing techniques are developing rapidly, and permit modifications such as gene knockout and knockin to be performed in various cell lines and animals. This review summarizes chromosome transfer-related technologies and the combined technologies of chromosome transfer and genome editing mainly for the production of cell/animal models of human disease and humanized animal models. Specifically, these include: (1) chromosome modification with genome editing in Chinese hamster ovary cells and mouse A9 cells for efficient transfer to desired cell types; (2) single-nucleotide polymorphism modification in humanized Tc mice with genome editing; and (3) generation of a disease model of Down syndrome-associated hematopoiesis abnormalities by the transfer of human chromosome 21 to normal human embryonic stem cells and the induction of mutation(s) in the endogenous gene(s) with genome editing. These combinations of chromosome transfer and genome editing open up new avenues for drug development and therapy as well as for basic research.

Chromosome transfer technology

Chromosomes can be transferred from donor cells to recipient cells using several techniques of chromosome transfer. Different research groups are developing chromosome transfer techniques involving microcell-mediated chromosome transfer (MMCT) [1, 2], and the transfection of chromosomes with liposome carriers [3].

Chinese hamster ovary (CHO), A9, and DT40 cells are mainly used as donor cells in MMCT (Fig. 1a) because they are capable of micronucleation with elongated inhibition of microtubules [4] in response to colcemid [1] or TN16 and griseofulvin treatment [5]. Microcells are isolated by the centrifugation of cells forming micronuclei. Those microcells contains one or a few chromosomes and can be fused with recipient cells using polyethylene glycol (PEG) [1]. Because the efficiency of PEG-MMCT is low, virus protein-mediated MMCT such as MV-MMCT and Retro-MMCT was developed to increase the efficiency of chromosome transfer [6, 7].

Liposome carriers can also be used to transfer chromosomes. In this process, naked chromosomes are isolated from cells that have been induced to undergo mitotic arrest with colcemid treatment. The chromosomes collected by sucrose cushion centrifugation can be transfected to recipient cells using liposome carriers [3]. However, the efficiency of this method is low, so it is less commonly used for chromosome transfer than MMCT.

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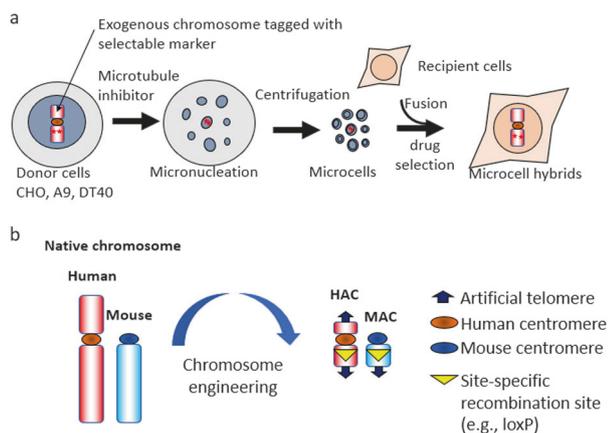3 Trans Chromosomics Inc., 86 Nishi-cho, Yonago, Tottori 683-8503, Japan

Fig. 1 Chromosome transfer technology and the top-down HAC/MAC. **a** Chromosome transfer technology: MMCT. Donor CHO, A9, and DT40 cells are exposed to a microtubule inhibitor leading to micronucleation. Microcells are then purified and fused to target cells. Microcell hybrids, target cells containing the desired chromosome, can be obtained. **b** Construction of the top-down HAC/MAC. HACs and MACs are stable in humans and mice, respectively

Applications of MMCT and the hurdles of conventional genome engineering technology

MMCT has been applied to various fields of molecular biology and biotechnology, e.g., chromosome mapping, functional assays associated with chromosome structure [2, 8], and the generation of transchromosomic (Tc) animals [2]. For example, an A9 human monochromosome library was used to transfer a target human chromosome via MMCT [9]. This library includes all human autosomes and X chromosome, which were deposited in the Japanese Collection of Research Bioresources Cell Bank [10].

Chromosome mapping is based on a complementation study [11, 12], and involves the introduction of a human chromosome via MMCT to rescue the phenotype of the model cells, such as cancer cell lines or disease model cells. This technique has been used for the identification of genes related to tumor suppression [13, 14], genomic imprinting [15, 16], DNA repair [17–19], metastasis and genomic instability [20], telomerase regulation [21–23], mitochondrial disorders [24], and lysosomal storage diseases [25]. MMCT has also been applied to investigate chromosomal functions such as kinetochore assembly, telomere function, and high-order chromosome architecture [8, 26, 27]. Tc animals harboring an extra copy of a human chromosome have also been generated via MMCT [28], including a Down syndrome (DS) mouse model [29, 30] and humanized immunoglobulin mice [31] and cattle models [32]. MMCT technologies and their applications are described in more detail in our previous review [2].

Although large DNA can be transferred using chromosome transfer technologies, difficulties remain using conventional genome engineering technologies for: (1) the efficient production of knockout (KO) animals for humanization and (2) the multiple and/or sophisticated genome manipulation required to improve existing human disease- and humanized animal-models, and to establish functional cell-based models. Therefore, the development of more efficient and convenient genome manipulation technology is urgently needed.

Genome editing techniques involving artificial nucleases mainly use zinc-finger nucleases [33], transcription activator-like effector nucleases [34], and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) [35]. These genome editing technologies allow KO or knock in cultures cells as well as animals to be produced. Details of the genome editing technologies are described in other reviews [36, 37]. Here, we describe the development of previously reported chromosome transfer-related technologies, and a new generation of chromosome transfer technology combined with genome editing techniques.

Combination of chromosome engineering and chromosome transfer

Chromosome modification in homologous recombination-proficient chicken DT40 cells

Two decades ago, chromosome modification relied on the occurrence of random events. Tc mice expressing the human immunoglobulin gene and genes on human chromosome 21 were generated with human chromosome fragments randomly obtained via MMCT [31] and X-ray irradiation-mediated MMCT [38], respectively. To perform required modifications without depending on random events, the homologous recombination (HR)-proficient chicken DT40 cell line, which has the capacity to undergo MMCT, was used for targeted gene insertion [15] and telomere-associated chromosome truncation to induce Mb-sized chromosomal deletions [39]. Such targeted chromosome modifications enable fine design of the chromosome and advanced applications to be made, e.g., the construction of mammalian artificial chromosome vectors including human artificial chromosomes (HACs) and mouse artificial chromosomes (MACs) (Fig. 1b), which can be used to generate humanized animals containing desired Mb-sized DNA.

Chromosome modification via genome editing in CHO and A9 cells

Although chromosome modification using HR-proficient chicken DT40 cells has enabled various unique
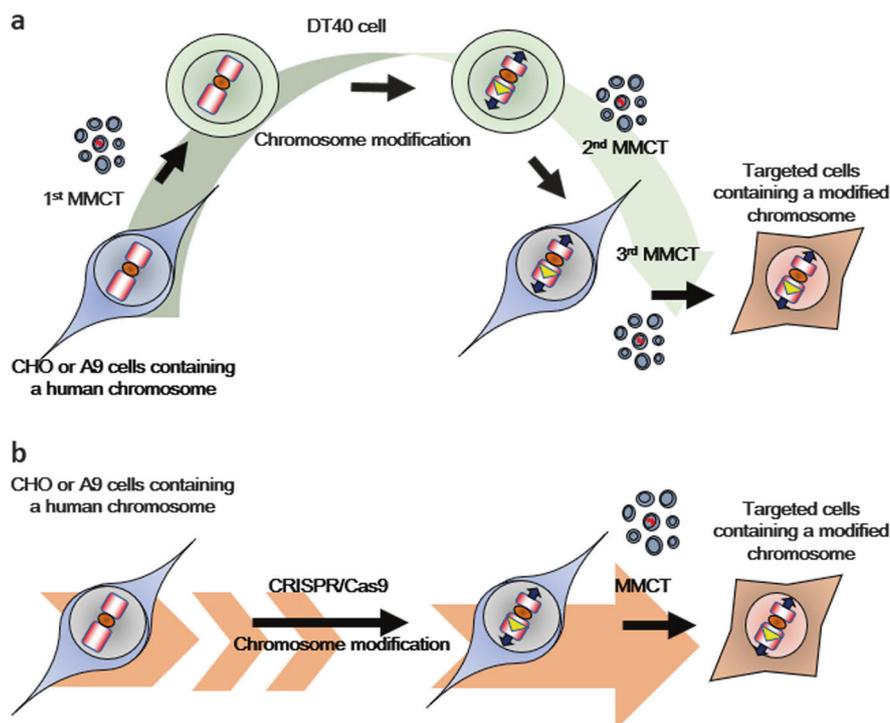
Fig. 2 Chromosome modification: gene insertion and telomere-associated chromosome truncation using CRISPR/Cas9 in CHO and A9 cells. **a** Diagram of conventional chromosome modification and the MMCT strategy. Three MMCT experiments are necessary to transfer the targeted chromosome to DT40 cells, re-transfer it to limited cell lines including CHO cells, and transfer it again to the targeted cells. This multistep of chromosome modification and MMCT is a detour. **b** Diagram of a new chromosome modification and MMCT strategy. The CRISPR/Cas9-mediated chromosome modification can be completed in CHO and A9 cells without the need for homologous recombination in DT40 cells, so the targeted chromosome can be directly transferred from CHO and A9 cells to the targeted cells

developments to be made, it first requires the transfer of a targeted human chromosome to DT40 cells and then re-transfer of the modified chromosome to CHO cells before transferring it to the desired cell type (Fig. 2a). Additionally, the donor cell type determines the MMCT efficiency, which is low when using DT40 cells, except for transfer to recipient CHO cells. Moreover, repeated MMCT is labor-intensive and time-consuming (Fig. 2a). Chromosome modification using CHO or A9 cells, but not DT40 cells, can avoid these problems, because repeated MMCT is not required. CRISPR/Cas9-mediated HR and telomere-associated chromosome truncation have recently been achieved in CHO and A9 cells, and the modified chromosomes were shown to be transferrable (Fig. 2b) [40]. Although the HR efficiency in CHO and A9 cells is lower than that of DT40 cells and chromosome modification requires negative selection with suicide genes or fluorescent markers [41] to eliminate cells harboring unexpected insertions of the modification plasmid vector, the CHO/A9 cell method has the advantage that the modified chromosome can be directly transferred to desired cells.

On the other hand, genome editing technologies are associated with two potential problems caused by the off-target effect [42]. The first occurs on the target chromosome although CHO and A9 cells only contain a single targeted human chromosome. It is therefore important to ensure that an off-target sequence is located on the targeted chromosome. The second occurs on the host chromosome of CHO or A9 cells. In this case, it is not necessary to identify an off-target effect because the modified chromosome will be transferred to other cells from the host cells. Thus, targeted cells transferred the modified chromosome avoid the risk of off-target effects by CRISPR/Cas9. This is a particularly valuable advantage of chromosome modification via genome editing in CHO and A9 cells than conventional genome editing in targeted cells without MMCT.

DT40-mediated chromosome modification techniques require ~6 months for three MMCT procedures to be completed, including 1 month for MMCT and cell cloning, and another month for PCR and chromosome analyses including fluorescence in situ hybridization analysis. In contrast, CHO- or A9-mediated chromosome modification techniques can be completed more quickly (~2 months), so have been able to reduce the start-up hurdles for chromosome engineering, including the transfer of Mb-sized chromosome loci.

HAC/MAC technologies

Characteristics of HACs/MACs

HACs have mainly been constructed in two ways, the bottom-up or top-down method. The bottom-up approach generates de novo HAC formation by component assembly in host cells [43–45]. One such HAC vector (tet-O HAC) with an artificial centromere sequence is conditionally removable from host cells, so is particularly useful for gene function analysis [46]. The top-down approach generates HACs from a native chromosome by chromosome engineering technology, including gene-targeting and telomere-associated chromosome truncation in DT40 cells [47–49] (Fig. 1b). It is also possible to construct an artificial chromosome from any mammalian chromosome or cell type using the genome editing techniques described above. Most bottom-up HACs are circular and their construction relies on spontaneous assembly. In contrast, all top-down HACs are linear, precisely engineered, and stably maintained in host cells. The bi-HAC system utilizing both top-down and bottom-up HACs, and benefitting from the advantages of both, was reported previously [50].
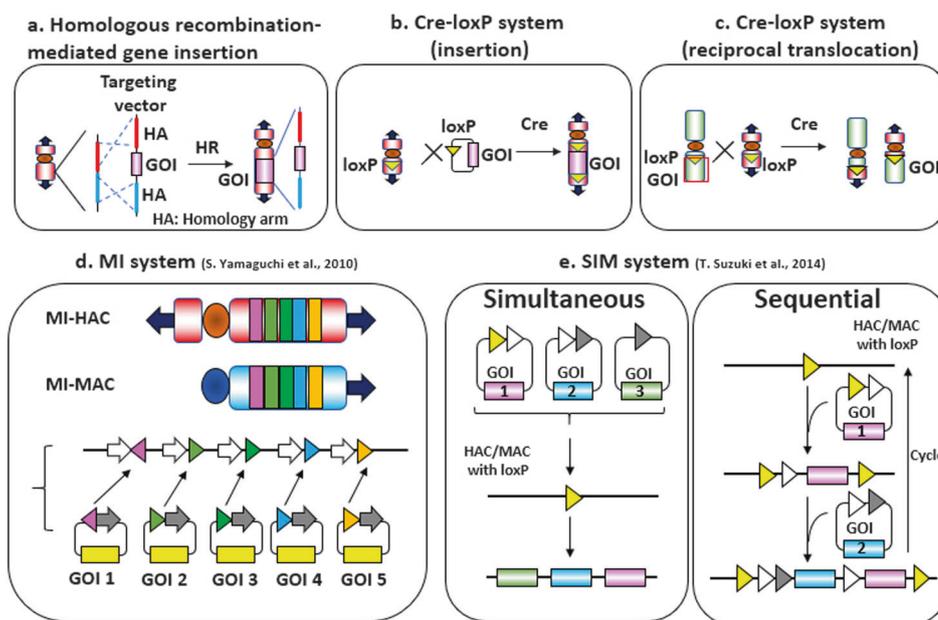
Regarding Tc animal production, the generation of Tc mice carrying HACs with gene(s) of interest [51–53] has been achieved by using MMCT to establish mouse embryonic stem (ES) cells containing the HACs. If germline-transmissible chimeric animals cannot be developed using ES cells, cloning technologies can instead be applied to generate Tc animals via MMCT to fibroblasts. This technology was used to generate Tc calves producing human immunoglobulin [32].

Although HACs are stable in human cell lines and some cells and tissues of other species, they have shown variable retention rates in mouse tissues, being especially low in mouse hematopoietic cells with high turnovers [54]. To overcome this problem, MACs were generated by the top-down approach to have the same capacity as HAC vectors with the additional advantage of highly stable maintenance in mouse tissues including hematopoietic cells. Therefore, MACs are more valuable gene delivery vectors for the production of model mice than HACs [54, 55]. Although several groups have developed MACs using different methods, to our knowledge those derived from native mouse chromosomes via the top-down approach are the most stable in mice [56, 57]. In summary, HACs/MACs as gene delivery vectors can deliver large genomes, and are stably and independently maintained with defined copy numbers in host cells, as well as being transferrable to desired cell lines via MMCT [2].

Gene-loading techniques for HACs/MACs

Five types of gene cloning system can be utilized with top-down HACs/MACs: (a) HR-mediated gene insertion; (b) the Cre/loxP-mediated insertion system; (c) the Cre/loxP-mediated translocation system; (d) the multi-integrase (MI) system; and (e) the simultaneous or sequential integration of multiple gene-loading vectors (GLVs) (SIM) system. In system (a), gene loading with unique sequences in HACs/MACs via HR in DT40 cells has previously been performed [49, 54] (Fig. 3a). The same gene-loading system involving HR in other cells such as CHO and A9, but not DT40 cells, was succeeded by genome editing technologies [40].

Fig. 3 Gene-loading methods applicable to HACs/MACs. **a** Homologous recombination-mediated gene insertion for gene of interest (GOI). **b** Cre/loxP-mediated circular DNA insertion. **c** Cre/loxP-mediated large genomic DNA fragment loading by reciprocal translocation. **d** The MI system. Five gene-loading vectors can theoretically be inserted into the MI system. **e** The SIM system. Three gene-loading vectors can be inserted simultaneously. The sequential method inserts gene-loading vectors one by one indefinitely. Both methods enable the theoretical indefinite integration of gene-loading vectors

HACs/MACs generally harbor loxP sites for gene loading. In system (b), circular vectors such as plasmids, P1 artificial chromosomes (PACs), and bacterial artificial chromosomes (BACs) were successfully loaded onto HACs/MACs by the Cre/loxP system for various purposes including functional analysis, monitoring system development, and gene therapy [58–64] (Fig. 3b). This loading system is particularly suited to when the gene of interest is covered by one of these circular vectors. In system (c), Mb-sized chromosome fragments such as the human CYP3A cluster and human dystrophin gene (~2.4 Mb) can be loaded onto top-down HACs/MACs [53, 65] (Fig. 3c). This system is suitable when the gene(s) of interest exceeds the delivery size limit of PACs and BACs. It involves manipulation of the chromosome containing the genomic region of interest by telomere-associated truncation and loxP targeting. The genomic region is eventually loaded on the HACs/MACs by Cre/loxP-mediated reciprocal translocation cloning in CHO cells.

Previously, the HAC/MAC system has mainly employed the Cre/loxP system in CHO cells for gene loading as mentioned above. However, two multiple gene-loading systems, the MI and SIM systems, have recently been developed for application in HACs/MACs with loxP sites [66, 67].

In system (d) (the MI system), four serine integrases and one recombinase (PhiC31, Bxb1, R4, TP901-1, and FLP) are utilized. Using all recombination sites, five GLVs can theoretically be inserted into the MI-HAC (Fig. 3d). For the efficient and convenient production of Tc animals carrying gene(s) of interest, a MAC carrying multi-integration sites (MI-MAC) was also constructed [54]. The MI-MAC has been transferred into mouse ES cells to enable gene(s) of interest to be directly loaded onto it [68]. The MI-MAC has also been transferred into HepG2 cells to verify cytotoxicity levels via luciferase reporter assay [69]. The MI-HAC/MAC system can be used in various cell types and has successfully achieved the insertion of large GLVs such as PACs and BACs and multiple genes into the MI-HAC/MAC [70–72]. Although limitations exist regarding the available drug-resistant genes for five GLVs loading onto the MI-HAC/MAC vector, this was solved by reusing the same drug-resistant genes by disrupting them with genome editing technology (Honma et al., 2017, unpublished data). This system is particularly valuable for gene loading when required number of gene loading is within five times and if the MI-HAC/MAC has already been transferred to the desired cell type.

In system (e) (the SIM system), two integrases and two recombinases (PhiC31, Bxb1, Cre, and FLP) are used. This system is applicable to HACs/MACs with loxP sites, and either simultaneous or sequential integration can be performed (Fig. 3e) [67]. The simultaneous integration system enables the simultaneous integration of three GLVs into HACs/MACs and further sequential insertions. The sequential integration system enables GLV loading one by one. Both methods theoretically allow the indefinite loading of GLVs onto HACs/MACs. Selection for gene insertion is performed by switching on/off hypoxanthine-guanine phosphoribosyl transferase (HPRT) reconstitution or neomycin resistance. Thus, gene loading onto HACs/MACs has to be performed in HPRT-deficient cells. Although gene loading with the SIM system is restricted in HPRT-deficient cells, HPRT KO in cells of interest can readily be performed by genome editing technology leading to direct gene loading onto HACs/MACs. The SIM system is particularly suitable when efficient simultaneous multi-gene loading or unlimited gene loading is required. HACs/MACs with MI or SIM systems are expected to be employed in a variety of fields and can be chosen depending on the purpose. Taken together, the combination of five cloning methods onto top-down HACs/MACs and genome editing will be useful for the cloning of desired genes and gene clusters. Regarding bottom-up HACs, systems (a, b, d, and e) are applicable to bottom-up HACs with loxP sites. However, bottom-up HACs cannot be used for reciprocal translocation-mediated gene cloning in system (c) because they are mostly circular and contain multiple loxP sites.

Humanized animal model: the CYP3A model mouse

The HAC/MAC system is a powerful tool for producing humanized animal models harboring multiple or large genes with physiological expression [2]. As an example, the humanized CYP3A mouse model was generated using HAC vectors [53]. CYP3A genes encode enzymes involved in drug metabolism and are associated with the metabolism of approximately 50% of commercially available drugs. However, CYP3A enzymes differ between species, so animal models such as the mouse and rat do not reflect human CYP3A-related pharmacokinetics. Therefore, humanized CYP3A model animals are useful for predicting CYP3A-related pharmacokinetics and toxicities of new drugs in humans.

CYP3A genes including CYP3A4, CYP3A5, CYP3A7, and CYP3A43 form a cluster on human chromosome 7. It is difficult to introduce large genomic regions into mice by conventional technologies, so to produce a humanized CYP3A mouse model, human chromosome 7 was modified in DT40 cells by chromosome engineering and the CYP3A cluster was cloned into a HAC vector (CYP3A-HAC). The CYP3A-HAC was transferred into mouse ES cells to produce chimeric mice that transmitted the CYP3A-HAC through the germline. Further crossing with Cyp3a-KO
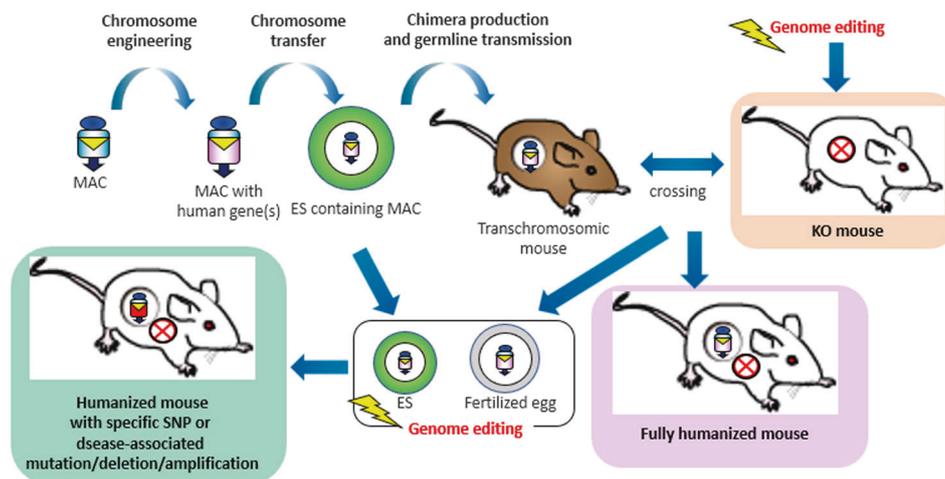
Fig. 4 Graphical summary for the generation of humanized model mice. The development of genome editing techniques has enabled the rapid modification of chromosomes in ES cells and fertilized eggs. To generate a humanized model, genome editing techniques permit the knockout (KO) of orthologous gene loci in the mouse genome. Crossing KO mice with transchromosomic (Tc) mice generate fully humanized mice. Genome editing techniques realize the further modification of human genomic loci on the MAC to produce humanized mice carrying specific single-nucleotide polymorphisms or disease-associated mutations, deletions, or amplifications for a personalized model

mice produced fully humanized CYP3A mice that showed gender-, tissue-, and developmental stage-specific *CYP3A* expression, similar to humans [53, 73]. The humanized CYP3A mice also recapitulated the CYP3A metabolic activity observed in humans. Furthermore, whole-embryo cultures of the humanized CYP3A mouse embryo showed teratogenic effects of thalidomide that are not seen in rodents [74]. Thus, humanized CYP3A mice are expected to be useful for drug screening by predicting CYP3A-related drug metabolism.

Such drug metabolism-related genes generally form large clusters, so chromosome engineering including HAC/MAC technologies and chromosome transfer are powerful technologies for producing humanized mice carrying these genes. Furthermore, other humanized models of the human immune system or particular genetic diseases are likely to be produced in the future.

Previously, KO of endogenous orthologous genes was laborious, especially in ES cells for fully humanized model production [53]. Gene cluster KO requires the deletion of a large targeted region, mainly using the Cre/loxP system, so is labor- and time-intensive as well as expensive because targeting of two loxP sites and Cre/loxP-mediated chromosomal deletion must be performed in mouse ES cells, and chimeric mice formation assays are required at each step. To overcome the problem of ES cell-based technologies, genome editing technologies can be utilized to induce mutations, deletions of single genes, and large genomic deletions for orthologous gene KO [75]. Now, the combination of chromosome transfer and genome editing technology is crucial for the efficient production of fully
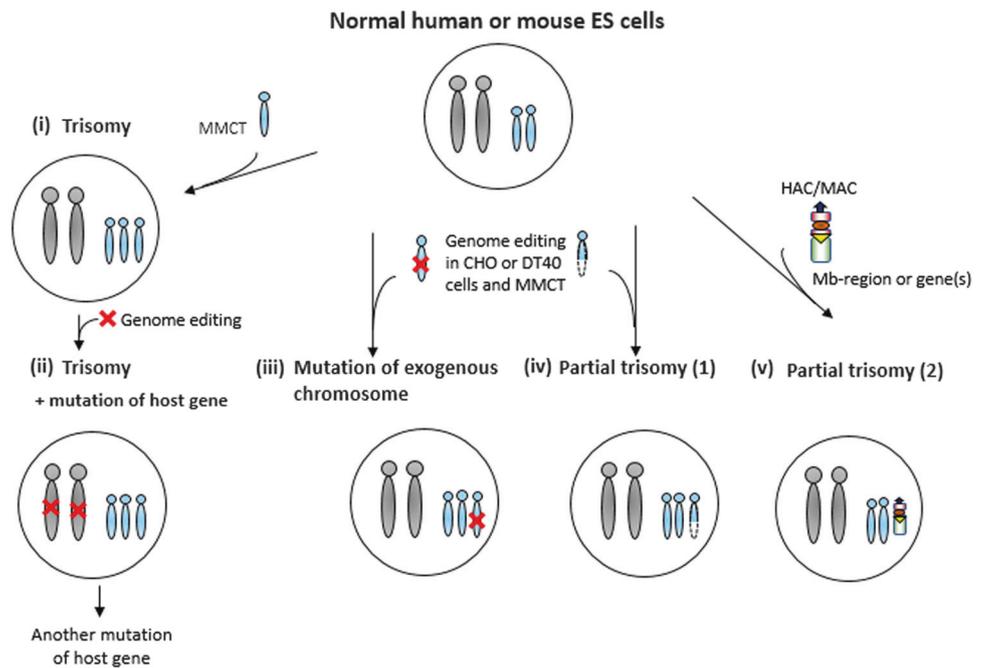
humanized animal models (Fig. 4) [76]. If genome editing of KO endogenous genes can be applied to germline-transmitted Tc animals, both time and cost for mating Tc and KO animals will be saved. Additionally, the combination of HACs/MACs and genome editing can be adapted to generate humanized model rats which offer several advantages over mouse models [77, 78].

Replacement of single-nucleotide polymorphisms in humanized animal models

Genome editing technologies enable the further modification of genomic sequences in pre-constructed HACs/MACs. Numerous single-nucleotide polymorphisms (SNPs) of human drug metabolism-related genes are reported to affect drug pharmacokinetics [79–81]. For example, *CYP3A5* SNPs have been reported in intron 3: CYP3A5*1(6986A) and CYP3A5*3(g.6986A>G) [82–84]. CYP3A5*1 allele carriers express CYP3A5 mainly in the liver and intestine, while homozygotes of the CYP3A5*3 allele lack almost all CYP3A5 protein expression [85].

Recently, an MAC was utilized to produce humanized CYP3A mice (CYP3A-MAC mice), and human-like CYP3A-related drug metabolism was observed in the CYP3A-MAC mice as well as in the CYP3A-HAC mice (Kazuki et al., 2017, unpublished data) [53]. However, the *CYP3A5* SNP genotype of humanized CYP3A mice was CYP3A5*3, and the mice lacked CYP3A5 protein expression. To produce humanized CYP3A mice that can be

Fig. 5 Graphical summary of a disease model of aneuploidy syndrome. Chromosome transfer techniques enable the generation of a disease model associated with aneuploidy via the following methods: **i** Transfer of an additional chromosome in the same genome background; **ii** the reproduction of advanced mutations on the host genome for research into associated gene (s) using genome editing; **iii, iv** transfer of chromosomes with mutations or truncated chromosomes generated by genome editing in CHO or DT40 cells; and **v** identification of responsible gene(s) or loci using HACs/MACs with Mb-regions or gene(s)

utilized to predict the CYP3A5 contribution to drug metabolism, the *CYP3A5* SNP on the CYP3A-MAC was modified from CYP3A5*3 to CYP3A*1 by CRISPR/Cas9 in both mouse ES cells carrying the CYP3A-MAC and fertilized eggs carrying the CYP3A-MAC [86, 87, 106]. Modification with CRISPR/Cas9 was achieved by the transfection of mouse ES cells carrying the CYP3A-MAC using double-stranded circular DNA containing a 1 kb region of homology with the SNP, and pronuclear injection with 135 bp single-stranded DNA with the SNP as the donor. The resulting CYP3A5*1 mice showed higher CYP3A5 protein expression in their liver and intestine compared with CYP3A5*3 mice, and CYP3A5 was found to contribute more to metabolism in CYP3A5*1 mice. These results suggested that the *CYP3A5* SNP had been functionally modified, and that the SNP effect could be recapitulated in mice because CYP3A5*1 mice showed the CYP3A5*1 carrier phenotype.

HACs/MACs can deliver entire gene regions with elements for physiological expression, and can maintain defined copy numbers, especially single copies, in model animals [53–55]. Therefore, modified SNPs can be more efficiently identified in Tc animals than in conventional genome-edited animals with diploid genomes. The combination of HAC/MAC systems and genome editing technologies is valuable for validation of the SNP effect in humans by producing humanized model animals with corresponding SNPs. Because genome editing technologies enable human genes to be efficiently modified on HACs/MACs, disease-associated mutations-, deletions-, and amplification-harboring models that reflect individual
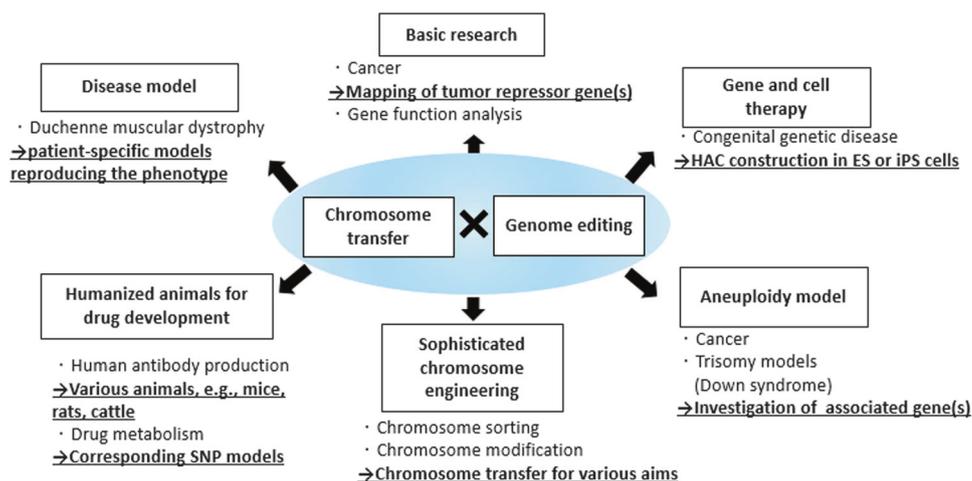
patient phenotypes can be generated from humanized animals (Fig. 4).

In vitro models for aneuploidy and cancer

Chromosome transfer is a powerful technology for producing aneuploidy syndrome cell models such as trisomy 21, 18, and 13, and cancer-initiating cell models to reflect the contribution of specific chromosome amplifications to cancer progression. One of the aneuploidy syndromes, DS, also known as trisomy 21 (Ts21), is the most common genetic chromosomal abnormality. Infants with DS suffer from transient abnormal myelopoiesis (DS-TAM) at a high frequency, and approximately 20–30% of cases subsequently develop DS-related acute megakaryoblastic leukemia (DS-AMKL) [88–90]. Mutations in *GATA1*, encoding the megakaryocyte transcription factor, resulting in the production of N-terminal truncated GATA1 (GATA1s) is associated with DS-TAM and DS-AMKL [91–93]. The combination of *GATA1* mutation(s) and constitutive Ts21 is the most likely cause of DS-TAM, and additional mutations are thought to result in DS-AMKL [94].

To understand the mechanism of the progression to TAM in DS patients, Ts21, GATA1s, and GATA1s/Ts21 human ES cells were generated by combining chromosome transfer and genome editing technologies [95] (Fig. 5i, ii). Notably, all ES cell lines generated were isogenic and genetically defined with the advantage of chromosome transfer. Zinc-finger nucleases were used for the targeted mutation of *GATA1* exon 2 in human ES cells (WT-ES), and human ES

Fig. 6 Future applications of the combination of chromosome transfer technology and genome editing

cells in which both alleles were mutated (GATA1s-ES) were successfully obtained. Human chromosome 21 was then transferred via MMCT to WT-ES and GATA1s-ES cells to generate Ts21-ES and GATA1s/Ts21-ES, respectively. ES-sac-mediated in vitro hematopoietic differentiation analyses using the DS models revealed that Ts21 and the *GATA1* mutations synergistically contributed to hematopoietic abnormalities. Although another group established isogenic cell lines via the spontaneous loss of chromosome 21 from induced pluripotent stem (iPS) cells derived from a DS patient, they reported the synergistic interaction of Ts21 and *GATA1* mutations mainly from an assessment of non-isogenic cell lines with a different genotype [96]. It is crucial to establish isogenic cell lines for disease models harboring chromosome abnormalities.

The DS models generated by the combination of chromosome transfer and genome editing technology are useful for studying how *GATA1* mutations associate with the onset of TAM in DS patients. Recently, genomic profiling suggested that several mutations contribute to the progression from DS-TAM to DS-AMKL [94]. Thus, GATA1s/Ts21-ES may be used to screen factors involved in DS-AMKL progression through introducing additional mutations into GATA1s/Ts21-ES cells by genome editing technologies (Fig. 5ii). The introduction of chromosome 21 with a partial deletion or specific gene mutation generated by genome editing technology into normal ES cells, corresponding to partial trisomy 21, will help us to screen the region or gene(s) responsible for the disease phenotype (Fig. 5iii, iv). Moreover, the transfer of HACs/MACs carrying specific loci or genes on hChr.21 will enable the identification of the gene (Fig. 5v).

Genome editing studies validating the contribution of recurrent mutations to colorectal carcinogenesis suggested that aneuploidy, as the gain of chromosomes such as 7, 13, and 20, may be associated with aggressive tumor phenotypes [97, 98]. Direct confirmation of colorectal cancer

exacerbation can be achieved by generating aneuploidy models by MMCT of such human chromosomes. The combination of the two technologies will be useful for the generation of in vitro chromosomal abnormality models with multiple genetic alterations for the functional analysis of cancer development.

Conclusions and future perspectives

Chromosome transfer using MMCT is a unique and powerful technology for the generation of genetically modified animals for humanization and disease models. The introduction of Mb-sized genomic loci is technically difficult and takes several years using conventional BAC/transgenic-based genetic modification in mouse ES cells [99, 100]. Genome editing techniques simplify the genetic modification and expand the applications. Moreover, the combination of chromosome transfer and genome editing offers a new generation of chromosome engineering.

Future prospects based on this combination are summarized in Fig. 6. Sophisticated chromosome engineering enables specific chromosomes to be monitored, leading to efficient chromosome sorting and transfer, and complex chromosome modification for various purposes. For example, CRISPR/Cas9 may be applicable to improve the efficiency of MMCT. Isolated microcells are a mixed population containing targeted and/or host chromosomes. The chromosome tagging system involving dCas9 [101] fused with fluorescence proteins may be useful in purifying microcells containing a targeted chromosome.

Considering the use of HACs in gene and cell therapy, to avoid the potential contamination of animal components and unknown viruses derived from fused microcells, HACs should be constructed in normal human cells such as iPS cells, and transferred without using cell lines of other species such as CHO, A9, or DT40 cells. Additionally, it will

be beneficial to develop novel techniques to perform MMCT from human cells to targeted human cells such as patient-derived iPS cells.

The humanization of mice and larger animals such as cattle, pigs, and cats will be streamlined by efficient orthologous gene(s) KO using genome editing, and the generation of various humanized Tc animal models may promote drug development and therapy [102–104]. Once HACs/MACs with gene(s) of interest are constructed, they can be transferred to various mammalian cells and maintained independently without integration into the host genome. The generation of disease model animals such as Duchenne muscular dystrophy (DMD) with various deletions or mutations of the human DMD genome recapitulating individual human patients supports our understanding of detailed disease mechanisms resulting in different phenotypes.

In addition to generating synergistic driver mutations by genome editing, aneuploidy models generated by chromosome transfer provide an important insight into the hallmarks of cancer. For basic research, the screening of potential tumor repressor genes or gene clusters including noncoding RNAs with chromosome mapping by chromosome transfer will be promoted by the precise manipulation of chromosomes through genome editing. Chromosome mapping technology also ensures physiological gene expression following single chromosome transfer to identify reliable candidates.

Taken together, the benefits of the combination of chromosome transfer and genome editing not only aid efficiency and the acceleration of research, but also promote novel approaches and new perspectives in the field, such as synthetic biology involving genome writing [105].

Compliance with ethical standards

Conflict of interest Dr. M.O. is CEO of, and a shareholder in, Trans Chromosomics Inc. The remaining authors declare that they have no competing interests.

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