Chromosome therapy: different approaches and current challenges

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Unbalanced chromosomal abnormalities are associated with alteration of the gene dosage, with subsequent disruption of normal chromatin contact patterns and genomic regulation. Chromosome therapy has been proposed as a therapeutic intervention for amending various chromosomal aberrations. Numerous *in vitro* methods have been erupted where the goal was to eliminate the effects of the altered gene dosage. This could be achieved through removal or silencing the extrachromosomal material from the cells in cases of different syndromic trisomes and chromosomal duplications or to replace the deleted genetic material in cases of pure deletion either terminal deletion or that leads to ring chromosome formation. There are also different proposed ideas to comprise other structural abnormalities within the targets of chromosome therapy. Very recently, research studies on chromosome transplantation have been presented aiming to deal with various X-linked disorders as well as different structural chromosomal abnormalities. It is remarkable that several concepts of the proposed designs are either inspired or explained from naturally existing mechanisms for adjusting cell cycle, for example, using X-Inactivation Specific Transcript gene for silencing the added chromosome, uniparental disomy, and trisomy rescue. Although all studied strategies are still at the cellular level, the results of these studies will unravel different complex interacting pathways between multiple deleted or duplicated genes and identifying appropriate targets for therapeutic interventions. Current clinical application is confronted by certain obstacles, including ethical and technical limitations. As most of the proposed strategies for chromosome therapy have to be performed on continuously dividing cells and ethical considerations impede germline intervention, hence, at this stage, chromosome therapy can only be considered as a plausible therapeutic strategy for somatic, actively dividing cells.

Keywords:
chromosome therapy, human artificial chromosome, induced pluripotent stem cells, trisomy rescue, uniparental disomy, X-Inactivation Specific Transcript gene

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Introduction

Various human genetic diseases are associated with gross chromosomal abnormalities that involve multiple genes in the aberrant chromosome. These abnormalities include deletions, duplications, and different types of aneuploidies with an incidence of ~1: 150 live births (Hsu, 1998). Ring chromosome is another chromosomal abnormality that is prevalent in about one in 50 000–100 000 (Bershteyn *et al.*, 2014) and is usually formed due to terminal deletion (Morgan, 1926).

These chromosomal abnormalities are usually associated with alteration of the gene dosage within the nucleus, which can disrupt the nuclear structure and organization, with subsequent affection of normal chromatin contact patterns and genomic regulation. As numerous genes are involved in this process, the deleterious effect could be augmented due to involvement of different genes in the same or linked pathways (Cremer and Cremer, 2001; Hancock, 2014).

Current available managing strategies are maintained for monogenic disorders through manipulating a single gene at a time, thus they are not beneficial for confronting chromosomal abnormality disorders (Naldini, 2015; Plona *et al.*, 2016).

Although targeting an alteration of a high gene dosage is a challenging approach, however, various techniques have been recently evolved in attempt to deal with these distressing disorders under the heading Chromosome Therapy (Li *et al.*, 2012; Kim *et al.*, 2014).

Chromosome therapy is a promising future therapeutic plan directed to alleviate the devastating effects of these large chromosomal abnormalities through amending numerous genes and regulatory elements of the aberrant chromosome. To achieve these goals, it is mandatory to develop methods for abolishing the abnormal chromosome and/or substituting it by a normal functioning one (Amano *et al.*, 2015). Hence,

numerous *in vitro* methods have been proposed for addressing different chromosomal abnormalities.

Literature review

Proposed techniques for implementing chromosome therapy

The goal is to remove or silence the extrachromosomal material from the cells or to replace the genetic material that is lost, thus eliminating the deleterious effects of the altered gene dosage.

It is noteworthy that several proposed designs are either inspired or explained from naturally existing mechanisms for modifying cell cycle errors (Table 1).

Using X-Inactivation Specific Transcript gene for silencing the added chromosome

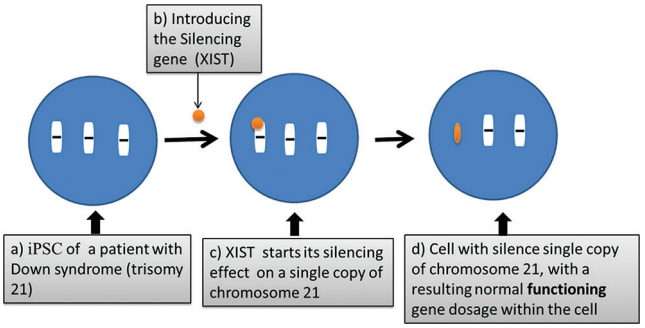
The X-Inactivation Specific Transcript (XIST) gene is a noncoding RNA gene normally located on the X chromosome and is responsible for silencing and inactivation of one of the two X chromosomes in females (Brown *et al.*, 1991). During embryonic development, the XIST RNA accumulates along the whole length of one X chromosome that becomes the inactive X with subsequent silencing of most genes. This silencing leads to dosage compensation between males (XY) and females (XX) (Clemson *et al.*, 1996).

Jiang *et al.* (2013) took the advantage of this normal mechanism for applying the same model using an inducible version of XIST to be inserted in induced pluripotent stem cells (iPSCs) derived from patients with Down syndrome (trisomy 21). The XIST was directed to a single copy of chromosome 21 by the aid of zinc-finger nucleases, this was followed by activation of the XIST gene using doxycycline treatment. This resulted in silencing of the additional chromosome with subsequent correction of the altered gene dosage. Moreover, the observation that cellular performance has been improved *in vitro* in the form of increasing cellular proliferation and neural differentiation, suggested that this approach could eventually lead to conclusive treatment for Down syndrome. Hence, this technique is considered a potential mechanism for silencing whole chromosomes in different types of trisomies and could also be used in cases of partial trisomies due to large duplications (Fig. 1).

Positive–negative selectable markers to abolish the extra chromosome

This technique was presented by Li *et al.* (2012), where thymidine kinase–neomycin (TKNEO) transgene was introduced through an adeno-associated viral vector in iPSCs obtained from a patient with Down syndrome. TKNEO transgene encodes both neomycin resistance and ganciclovir sensitivity (Chan *et al.*, 2001). So, when the whole set of cells was treated with a toxic neomycin analog (G418), cells devoid of the TKNEO transgene were excluded from the cohort (positive selection). Only cells that containing the transgene were proliferated. Consequently, a cohort of disomic cells started to appear, then all cells were treated with ganciclovir, so any aneuploid cells containing the TKNEO transgene were eliminated (negative selection). The end result was a subpopulation of pure disomic cells (Fig. 2).

Figure 1



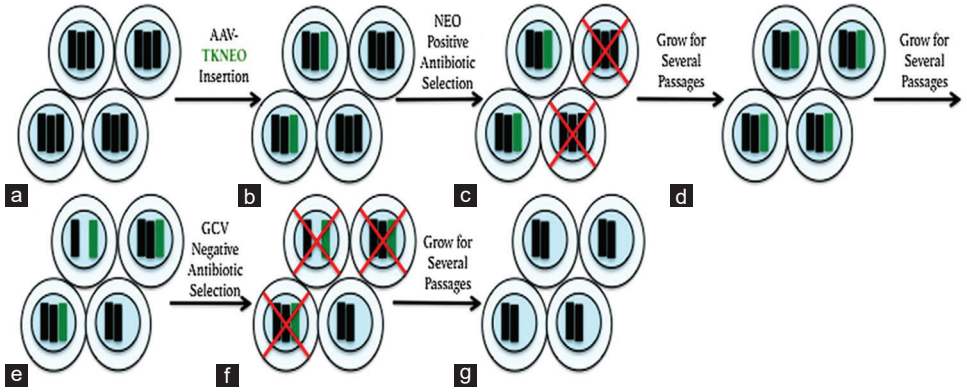
Using X-Inactivation Specific Transcript (XIST) gene for silencing the added chromosome. XIST is inserted in induced pluripotent stem cells (iPSCs) derived from patient with Down syndrome to induce silencing of the additional chromosome.

Table 1 Association between proposed techniques for chromosome therapy and the naturally existing mechanisms

Technique	Naturally existing mechanism
Using XIST gene for silencing the added chromosome in different types of trisomies	The XIST gene is a noncoding RNA gene normally located on the X chromosome and is responsible for silencing and inactivation of one of the two X chromosomes in female mammals
Using ring chromosome to improve different chromosomal abnormalities	Duplication of the normal homolog after losing the ring chromosome occurs through uniparental disomy (proved explanation)
(a) Correction of ring chromosome by excluding it from the cell cycle through cellular autonomous correction	Modifying the ring chromosome through double strand break and mitotic homologs recombination (suggested explanation)
(b) Induce ring formation of the aberrant chromosome by Cre-lox recombination technology to be excluded from the cell cycle	
ZSCAN4-induced trisomy rescue	Enhancement of the euploid sate in the culture simulating trisomy rescue
Chromosome transplantation	Spontaneous loss of the extra chromosome through trisomy rescue.

XIST, the X-Inactivation Specific Transcript gene; ZSCAN4, zinc finger and SCAN domain containing 4 gene.

Figure 2



Positive–negative selectable markers to abolish the extra chromosome (Plona *et al.*, 2016) (a) thymidine kinase–neomycin (TKNEO) transgene was introduced in the induced pluripotent stem cells (iPSCs) from a patient with Down syndrome. (b) The set of cells was treated with a toxic neomycin analog (G418). (c) Cells devoid of the TKNEO were excluded (positive selection). (d) Cells containing the TKNEO got proliferated. (e) Disomic cells started to appear. (f) All cells were treated with ganciclovir (GCV), cells containing the TKNEO were eliminated (negative selection). (g) A subpopulation of pure disomic cells starts to appear

Using ring chromosome to improve different chromosomal abnormalities

Ring chromosomes usually result from breaks at the P and q ends of the chromosome with subsequent union of the broken ends to produce a continuous ring. Two approaches involving ring chromosome have been proposed for chromosome therapy.

Correction of ring chromosome by excluding it from the cell cycle through cellular autonomous correction

Bershteyn *et al.* (2014) studied derived fibroblast cell lines from a patient with Miller–Dieker syndrome, deletion of 17p13.3 in this patient led to formation of a ring chromosome 17. Reprogramming of these fibroblasts into iPSCs resulted in a cellular autonomous correction, where the ring chromosome was lost and the normal homolog was duplicated.

Two different mechanisms have been suggested to elucidate these results, either uniparental disomy (UPD) with loss of heterozygosity where both chromosome homologs were derived from a single parent (Robinson, 2000) or the breakage of the ring followed by repair through mitotic homologous recombination (Moynahan and Jasin, 2010).

For distinguishing between these two possibilities, single-nucleotide polymorphism array has been performed to determine the degree of homozygosity on chromosome 17.

The results of single-nucleotide polymorphism array supported the mechanism of UPD. All these observations suggest that ring chromosome formation could be a feasible approach to abolish chromosomal aberrations associated with ring chromosomes with further improvement of the clinical phenotype.

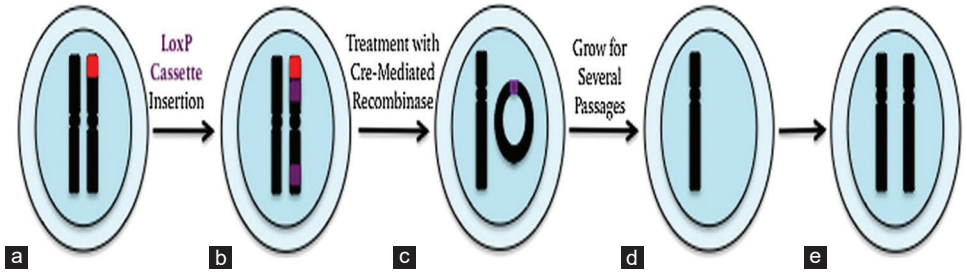
Induce ring formation of the aberrant chromosome by Cre-lox recombination technology to be excluded from the cell cycle

Plona *et al.* (2016) assumed that mimicking the previous natural mechanism of chromosome loss and compensation, amending different structural chromosomal abnormalities can occur, as the aberrant chromosome can be manipulated to form ring chromosome with subsequent removal from the cell line during cellular proliferation. Rapid proliferation of the iPSCs containing the ring chromosome could lead to its substitution with a normal copy through UPD. This can be performed by using site-specific Cre-lox recombination technology (Van Duyne *et al.*, 2015), where loxP (34-bp directionally oriented sequences) is inserted in both arms of the abnormal chromosome (p and q), then Cre recombinase enzyme will cause recombination between the two loxP sites, and the genetic material in-between will be lost to form a ring. Cells will proliferate for several passages to allow for losing the ring chromosome (Fig. 3).

Zinc finger and SCAN domain containing 4-induced trisomy rescue

Amano *et al.* (2015) implemented a trial in cultures of Down syndrome and Edwards syndrome (Trisomy 18) patients' fibroblast cells. They used zinc finger and SCAN domain containing 4 (ZSCAN4) gene, which encodes a protein involved in telomere maintenance and sustaining normal karyotype for sequential cell divisions in the culture (Zalzman *et al.*, 2010). To deliver ZSCAN4 inside the cells, they used integration-free Sendai viral vector (SeV-hZSCAN4) or synthetic mRNA (Syn-hZSCAN4) encoding ZSCAN4. Transient expression of the ZSCAN4 protein resulted in an increase of disomic cells in the culture, where high resolution G-banding technique revealed 40% normal disomic cells.

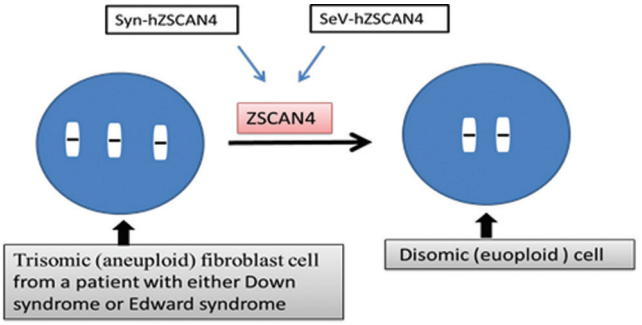
Figure 3



Using Cre-lox recombination technology to form ring chromosome with subsequent removal from the cell cycle (Plona *et al.*, 2016). (a) Induced pluripotent stem cells (iPSCs) from a patient with structural chromosomal abnormality. (b) LoxP are inserted in both arms of the abnormal chromosome. (c) Cre recombinase enzyme will cause recombination between the two loxP sites, the chromatin in-between will be lost to form ring. (d) Cells will proliferate to allow losing the ring chromosome. (e) Copying of the normal homolog.

Losing of the extra chromosome is suggested to occur through a mechanism simulating the natural existing mechanism termed trisomy rescue (Amano *et al.*, 2015) where normally if a fertilized ovum contains three copies of a chromosome, it can lose one of these chromosomes to form a normal, diploid chromosome complement (Balbeur *et al.*, 2016). Hence, without direct genetic alteration, induction of this small biologic molecule was able to enhance an euploid state in the culture and allow for selection of disomic cells (Fig. 4).

Figure 4



ZSCAN4-induced trisomy rescue. Using ZSCAN4 protein resulted in an increase of disomic (euoploid) cells in the culture. ZSCAN4, zinc finger and SCAN domain containing 4.

Human artificial chromosomes for replacing deleted genes

Normally human centromere chromatin and heterochromatin are assembled as a centromeric highly repetitive DNA sequence called alphoid DNA or α-satellite DNA. α-satellite DNA can form a de novo centromere and subsequent human artificial chromosome (HAC) when introduced into the human culture of fibrosarcoma cells. HACs are considered as microchromosome that can act as a vector for gene transfer (Ohzeki *et al.*, 2020), HAC as a vector could transfer the entire human dystrophin gene (2.4 Mb) that were stably maintained in mice and human immortalized mesenchymal stem cells.

HACs have several advantages over other gene delivery vectors, as they have unlimited transgene capacity compared with bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC), which have transgene capacity of about 300 kb and 2.5 Mb, respectively. YAC are genetically engineered chromosomes derived from the yeast DNA, where a sequence of DNA could be inserted and cloned, whereas BAC is an engineered bacterial DNA molecule used for DNA sequences cloning (Kazuki and Oshimura, 2011).

The genetic material introduced by BAC and YAC vectors can cause alteration of the expression levels and can disrupt the original human genome. HACs differ in this regard, as they are naturally segregates in human cells with neither integrating nor immunogenic consequence (Kouprina *et al.*, 2014). Moralli and Monaco (2015) reported that HACs have been shown to form functional kinetochores, which maintain their stability through successive cellular divisions, and even through differentiation of human embryonic stem cells to multiple cell lines. Although HACs are stable intracellular, they are very fragile, extracellular, and always require a suitable technique for intracellular delivery.

Accordingly, Plona *et al.* (2016) suggested that using HACs could be ideal for reverting chromosomal deletions. Where this can provide stable and functional copies of the deleted genes, as well as secure expression of the genes in the typical physiological pattern for each cell type receiving a copy. However, the availability of a suitable technique is mandatory to allow intracellular transmission.

Chromosome transplantation

Chromosome transplantation (CT) is a recent approach that could be categorized under the field of chromosome therapy. CT could be defined as the substitution of an aberrant chromosome with an exogenous normal one (Castelli *et al.*, 2019). Paulis *et al.* (2020) declared the success of CT in

human iPSCs derived from Lesch–Nyhan syndrome patients (LN-iPSCs). LN syndrome is an X-linked genetic disorder characterized by a mutation in the HPRT gene (Nyhan *et al.*, 1996). Thus, the aim was to exchange HPRT-defective X chromosome with a normal one.

Initially, they have prepared a donor normal cell line that could be used for the correction of LN-iPSCs, then the normal donor X chromosome has been integrated with the LN-iPSCs through modified microcell-mediated chromosome transfer technique where a coalescing virus (EnvΔRvirus) was added to maintain fusion between the microcells and LN-iPSCs. The resulting fused cells were selected using hypoxanthine–aminopterin–thymidine medium to detect those in which the normal donor X chromosome has been successfully transplanted.

The selected cells with the additional X chromosome were liable for losing it spontaneously after several passages in the culture. The outcomes were normal diploid clones in which the endogenous aberrant chromosomes have been lost. Liability for spontaneous loss of the aberrant chromosome from LN-iPSCs could be ascribed to the naturally existing mechanism termed trisomy rescue (Li *et al.*, 2017; Inoue *et al.*, 2019). Paulis *et al.* (2020) accentuated that CT could be a therapeutic strategy for various X-linked disorders and assumed its efficiency in the adjustment of different structural chromosome abnormalities including large deletions.

Anticipated clinical applications and future prospects

Despite being at the research phase, chromosome therapy is a promising field as its clinical application could improve patients with different disorders associated with chromosomal abnormalities (Table 2).

Current obstacles for applying chromosome therapy

Ethical considerations

Chromosome therapy is limited by certain ethical issues; as current ethical implications impede germline genetic alterations, chromosome therapy will be limited to somatic cells in the near future. Confining chromosome therapy to the somatic cells will bear the chance of inheriting the abnormal chromosomes to the off springs through the untreated aberrant germ cells. Although germline intervention could preclude such inheritance, it may affect fetal development in unexpected ways or lead to long-term side effects that are not comprehended. Consequently, this will represent serious psychosocial implications at the personal and societal levels (Araki and Ishii, 2014; Ishii, 2015).

Technical limitations

Although several strategies have been proposed for chromosome therapy nevertheless, nearly all of them have certain technical obstacles that hinder their current clinical application. As chromosome therapy is worth, different studies tried to present suggestions to overcome these obstacles (Plona *et al.*, 2016; Paulis *et al.*, 2020) (Table 2).

Other general limitations for applying chromosome therapy include nonselective targeting, defective efficiency, potential immunological reactions, as well as cellular toxicity (Naldini, 2015). Owing to these limitations, there are some recommendations for restricting such type of therapy for tissues that can be isolated from the patient, where the defective tissue is isolated, amended *ex vivo* in controlled lab conditions, and then retransplanted into the same patient. Clinical utility and patient suitability are confirmed before transplantation. Bone marrow transplantation is one of the most common examples for this process (De Ravin *et al.*, 2016).

Besides, chromosome therapy cannot be applied to cells and tissues that are not actively dividing, for example, the postmitotic neurons of the brain, as most of the current suggested procedures require successive frequent cellular divisions. Hence, at this stage, chromosome therapy can only be considered as a plausible therapeutic strategy for somatic, actively dividing cells (Plona *et al.*, 2016).

Conclusions

Chromosome therapy has expanded the span of genetic disorders that can be manipulated to comprise chromosomal aberrations. Various techniques were developed aiming to remove or silence the extra chromosomal material from the cells or to replace the lost genetic material, thus eliminating the deleterious effects of the altered gene dosage. As all the studied strategies of chromosome therapy remain at the cellular level, animal experiments are expected to provide substantial contributions in this field, most importantly, genetically engineered mouse models including transgenic, knockout, and knock-in mice. This could allow testing the effect of this therapeutic intervention, hence facilitating the initiation of clinical applications.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Table 2 Potential clinical applications and technical limitations for chromosome therapy

Technique	Potential clinical applications	Technical limitations and suggested ideas to resolve	Reference
Positive-negative selectable markers on the extra chromosome	Different types of trisomies	Using adeno-associated virus as a viral vector is not reliable for selective and equal delivery to all cells (Naldini, 2015)	Li *et al.* (2012)
Using XIST gene for silencing the added chromosome	Different types of trisomies	This technique requires continuous treatment of patients with doxycycline inducible promoter to maintain activation of XIST and ensure continuous chromosome silencing. To overcome this problem, the technique should be modified to use a constitutively active promoter (Plona *et al.*, 2016)	Jiang *et al.* (2013)
	Proposed for chromosomal duplications[a]	Some genes could escape XIST-mediated silencing so, the effects of these genes may lead to genomic dysregulation with undesired consequences. Complete removal of the excess chromosome would be a more effective strategy (Letourneau *et al.*, 2014)	
Using ring chromosome to improve different chromosomal abnormalities			
(a) Correction of ring chromosome by excluding it from the cell cycle through cellular autonomous correction	Different genetic disorders associated with ring chromosome formation	As UPD could substitute the lost ring chromosome, adverse outcomes owing to homozygosity of a recessive mutation may occur. Thus, recessive mutations have to be carefully monitored in each individual patient before applying this intervention	Bershteyn *et al.* (2014); Plona *et al.* (2016)
(b) Induce ring formation of the aberrant chromosome by Cre-lox recombination technology to be excluded from the cell cycle	Proposed for different structural chromosomal abnormalities[a]		
ZSCAN4-induced trisomy rescue	Different types of trisomies	This technique requires lifelong treatment to maintain continuous expression of ZSCAN4 protein (Plona *et al.*, 2016)	Amano *et al.* (2015)
Human artificial chromosomes for replacing deleted genes	Proposed for chromosomal deletions[a]	HACs are fragile and liable for rapid degradation when extracellular, thus their delivery rely on MMCT which can affect the genome integrity. With improvement of the delivery techniques, using HACs will be ideal for reverting chromosomal deletions	Plona *et al.* (2016)
Chromosome transplantation	Several X-linked disorders.	Comparable to the use of HACs, CT requires MMCT for its application, which could affect the genome integrity	Paulis *et al.* (2020)
	Proposed for chromosomal deletions, duplications and inversions[a]	CT is linked to the epigenetic regulations of the transplanted chromosome, which could differ from that of the recipient	

[a]Suggested ideas that have not been performed at the research level. CT, chromosome transplantation; HACs, human artificial chromosome; MMCT, microcell-mediated chromosome transfer; UPD, uniparental disomy; XIST, X-Inactivation Specific Transcript; ZSCAN4, zinc finger and SCAN domain containing 4.

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