The fusion of the short (p) and long (q) arms of a chromosome is referred to as a “ring chromosome.” Ring chromosome disorders occur in approximately 1 in 50,000–100,000 patients. Ring chromosomes can result in birth defects, mental disabilities, and growth retardation if additional genes are deleted during the formation of the ring. Due to the severity of these large-scale aberrations affecting multiple contiguous genes, no possible therapeutic strategies for ring chromosome disorders have so far been proposed. Our recent study (Bershteyn et al.) using patient-derived fibroblast lines containing ring chromosomes, found that cellular reprogramming of these fibroblasts into induced pluripotent stem cells (iPSCs) resulted in the cell-autonomous correction of the ring chromosomal aberration via compensatory uniparental disomy (UPD). These observations have important implications for studying the mechanism of chromosomal number control and may lead to the development of effective therapies for other, more common, chromosomal aberrations.

General Clinical Features of MDS

Lissencephaly, or “smooth brain,” is a severe brain malformation that results from defective neuronal migration and likely defective neurogenesis. These patients display abnormal cortical layering, absent cortical folding, and enlarged ventricles. As a result, they also present with severe neurological abnormalities including mental retardation and intractable epilepsy. Often, lissencephaly is the only abnormality found in an individual, which is called isolated lissencephaly. In other cases, patients display lissencephaly and other abnormalities, which would be designated as a syndromic form of lissencephaly. One type of syndromic lissencephaly is Miller Dieker Syndrome (MDS). MDS patients display a severe form of lissencephaly, where the brain is completely smooth (agyria), and have characteristic craniofacial dysmorphisms.1,2 More than 30 years ago, the first MDS patient that had a chromosomal abnormality was found to have ring chromosome 17 (r17), which led to the identification of 17p as the site responsible for MDS due to loss-of-function across multiple genes on this chromosome arm.1 MDS is always caused by heterozygous deletions of the subtelomeric p-arm band of ch17(17p13.3).1,3 Consistent with this, the phenotype of the patient with r17 was similar to the phenotype of patients with large terminal deletions without ring chromosomes.

Molecular studies in patients with isolated lissencephaly without deletions of 17p identified a gene, LISI (now called PAFAH1B1) as the major gene in 17p13.3 responsible for lissencephaly.3 Other studies identified a critical MDS region...
encompassing PAFAH1B1 and extending toward the telomere to include the gene YWHAE, encoding the protein 14-3-3ε.4 Mouse models have confirmed the roles of PAFAH1B1 and YWHAE in neuronal migration, which is considered to be the main cellular deficiency in lissencephaly.5 To study the cellular and molecular mechanisms of human MDS pathogenesis and examine gene function in the deleted locus, we generated induced pluripotent stem cells (iPSCs) from three MDS patients. One of these patients was the original MDS patient with r17, and two other patients had typical >2 Mb deletions of 17p13.3 without r17.

Reprogramming of Patients-Derived iPSCs

iPSCs were generated by a transient overexpression of the Yamanaka factors in patient-derived fibroblasts. Specifically, we used episomal vectors to reprogram fibroblasts into iPSCs to avoid the genome integration of those transcription factors.6 The reprogramming efficiencies of fibroblasts from all three patients were similar. Multiple clones from all three patients were indeed pluripotent and validated as iPSCs. Next, we wanted to confirm the maintenance of the MDS deletion in iPSCs before conducting our phenotypic experiments. These studies included karyotyping, fluorescence in situ hybridization (FISH), and the examination of the expression of multiple genes in the deleted locus on chromosome 17. Two independent iPSC clones of each of these patients were analyzed for DNA, RNA, and protein expression of PAFAH1B1 and YWHAE, two genes that are deleted in all MDS patients. Each of the iPSC clones from the two MDS patients with deletions only were found to contain only one copy of PAFAH1B1 and YWHAE, as well as 50% reductions in mRNA and protein products from these genes (Fig. 1).

Figure 1. iPSC cells from a patient with r17 end up with two intact chromosomes 17 after reprogramming.

Loss of Ring Chromosome through Reprogramming

These surprising findings indicated that the ring chromosome 17 with a deletion was replaced by a normal chromosome 17 during the process of reprogramming, restoring normal copy number and the expression of the genes deleted in MDS. In contrast, the two other MDS patient lines with typical deletions maintained their deletions after reprogramming, indicating that it was not the deletion, but the ring itself that led to the loss of r17. To confirm the loss of ring chromosomes in MDS r17 iPSCs, we examined the composition of chromosomes in additional clones derived from MDS r17 fibroblasts. All stable iPSC clones had normal karyotypes, while unstable clones that did not result in the formation of iPSCs rarely had a normal karyotype. Interestingly, we never found r17 in metaphase spreads of stable iPSCs, which suggests that iPSCs with ring chromosomes may be non-dividing.

This phenomenon was generalizable to other chromosomes. We reprogrammed fibroblast from two additional cases containing ring chromosome 13 (r13), with large deletions. Stable iPSCs derived from these fibroblast lines displayed normal karyotypes without r13. Thus, it appears that reprogramming fibroblasts with both ring chromosomes (r17 and r13) into iPSCs eliminates the ring chromosomes, replacing the ring with a normal copy of the chromosome.

Two Possible Mechanisms of Ring Chromosome Rescue: Compensatory Uniparental Isodisomy (UPD) vs. Partial Loss of Heterozygosity (LOH)

We next investigated how reprogramming into iPSCs eliminates ring chromosomes. We considered two possible mechanisms. First, the entire ring chromosome is lost during reprogramming, and the entire chromosome is replaced by duplication of the remaining normal
chromosome, a process called uniparental isodisomy (UPD, Fig. 2). Alternatively, a double strand break of the ring chromosome forms a linear chromosome, and the wild type homolog is used as a template to repair the deleted locus of the chromosome by mitotic homologous recombination (Fig. 2). To distinguish between these two possibilities, we performed SNP arrays to determine the degree of homozygosity on chromosome 17 and 13 in iPSCs derived from the patients with r17 or r13, respectively. If the UPD model is correct, then only the region of 17 or 13 deleted in the ring chromosomes should be homozygous. The result of SNP arrays supported the compensatory UPD model, since the two homologs of chromosome 17 and 13 were completely homozygous in the r17- and r13-derived iPSCs. Therefore, the loss of the ring followed by duplication of the remaining chromosome resulting in UPD is the mechanism for the rescue of the ring chromosome with a normal copy of its homolog. We speculate that the karyotypically normal iPSCs with isodisomy for the corrected chromosome outcompeted co-existing aneuploid populations, allowing rapid and efficient isolation of patient-derived iPSCs devoid of the original chromosomal aberration.

Ring Chromosome Rescue in Vitro and in Vivo

The loss of ring chromosomes during reprogramming of iPSCs and the repair of ring chromosomes via UPD is likely the result of one of the normal homeostatic mechanisms that maintain genome stability and control the number of chromosomes in the human genome.7 Consistent with our observation of cell-autonomous correction of ring

Figure 2. Two potential mechanisms for the loss of r17 including the loss of the ring and replacement with a wild type homolog via the compensatory UPD (top) and the breakage of the ring with repair by mitotic homologous recombination (bottom).
chromosomes in vitro, ring chromosome correction in vivo has also been observed. For example, a patient with a diagnosis of ring chromosome 21 soon after birth was found several years later to have the majority of her lymphocytes with 46,XX,r(21) replaced with lymphocytes containing a 46,XX karyotype.8,9 It is also possible that this patient had somatic mosaicism in her bone marrow, where some cells were 46,XX,r(21) and others were 46,XX, and the rescue resulted from expansion of the normal 46,XX cells.

By contrast, the initial MDS patient with r17 appeared to have a stable r17 karyotype in all of his cells, since all cells in his blood contained r17 (Dobyns et al.), all fibroblasts had r17 (Bershtein et al.) and imaging studies of the brain revealed complete agyria, consistent with a uniform r17 MDS phenotype. Why, then, wasn’t r17 corrected in vivo, similar to what we observed in vitro? We reason that during reprogramming iPSC cells undergo a lot more highly stereotyped and rapid cell divisions than similarly staged ES cells in the developing embryo. In these rapidly dividing cells mitotic checkpoint systems are less efficient, leading to frequent nondisjunction of sister chromatids.10 In addition, ring chromosomes are known to be unstable due to failure to pair with its homologous chromosome, as well as the formation of anaphase bridges.10 This leads to frequent ring loss and production of monosomic cells. Thus, simultaneous ring loss and nondisjunction for the homologous wild type chromosome will give rise to an isodisomic cell that has a growth advantage over cells with chromosomal aneuploidy or aberrations.

Furthermore, there may be undesirable consequences of UPD in vivo since UPD may uncover homozygosity for recessive mutations or disruptions.12 Thus, actively and continuously proliferating cells such as human iPSCs in vitro are under more selective pressure to correct ring chromosomes than the in vivo situation.

**Potential Use of “Chromosome Therapy” via Cell-Autonomous Correction**

One promising application of iPSC technology is the potential use of patient-derived cells to correct defects by transplantation via regenerative medicine technology. If a patient has a monogenic disorder, gene therapy or new gene-editing techniques can be used to correct the genetic defect in these cells prior to transplantation.14 However, the use of stem cell therapy after gene correction is currently limited to monogenic diseases with an altered or mutated gene.14 In the case of multigenic disorders, a large number of gene modifications may be needed to alleviate the phenotype of diseases. Current gene-editing techniques are not feasible for large chromosomal abnormalities such as trisomies (additional copies of entire chromosomes) or large chromosomal aberrations such as deletions or duplications. Approximately 1 in 150 newborn infants are born with such chromosomal abnormalities.15 One recent study of iPSCs derived from patients with trisomy 21 demonstrated that the insertion of an inducible version of Xist, containing the X inactivation center, into one of the three copies of chromosome 21, results in inducible silencing of the chromosome and correcting the dosage imbalance.16 This exciting study was the first example of “chromosome therapy,” and provides a potential mechanism for silencing whole chromosomes in the case of aneuploidy, or numerical aberrations. However, whole chromosome silencing cannot be used for chromosomes with large deletions or duplications.

In addition to correction of ring chromosomes, our results suggest a novel approach to “chromosome therapy” for the correction of large chromosomal aberrations, which cannot be addressed using standard genome-editing techniques or Xist-mediated inactivation of whole chromosomes.14 We propose that one could induce the formation of
ring chromosomes from chromosomes that contain large aberrations, such as deletions or duplications. Thus, it will be important to examine whether it is possible to achieve the induction of ring chromosome for curing large chromosomal aberrations via a cre-loxP system, for example. This will result in the elimination of the induced ring chromosome and rescue with the wild-type chromosome via the compensatory UPD (Fig. 3). One possible complication is that the “normal” chromosome homolog may contain recessive mutations or the chromosome may be imprinted, leading to loss-of-function or dosage imbalance phenotypes. Genome-editing technologies can be used to correct these dosage imbalances when they occur. Therefore, our findings have the potential to lead to the development of therapeutic options to relieve the phenotypic symptoms of general chromosomal abnormalities in humans.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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