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Knocking out the bad allele

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When R Jaenisch was pioneering a technique to inactivate genes in transgenic mice by random retroviral insertion of Moloney murine leukemia virus, 30 years ago, one integration event occurred into the first intron of the murine procollagen α1 (I) gene (*Col1a1*), generating a null allele.[1] While homozygous transgenic mice lacked procollagen I expression and died during early development, heterozygous mice were affected by an extremely mild form that did not impair their reproduction and allowed the generation of the first knockout mouse line, noted as *Mov13*.

In a recent paper in *Science*,[2] DW Russell reports that an adeno-associated virus (AAV), carrying the DNA sequence of the first exon of human procollagen gene (*COL1A1*) with a neomycin resistance gene (neo^R) interruption, can target with very high efficiency the homologous region in human genome and knockout one *COL1A1* allele. The selection of neo^R clones showed an elevated targeting frequency in mesenchymal stem cells (MSC) from patients affected by osteogenesis imperfecta (OI). The inactivation of the allele with the mutation gave a substantial improvement to the procollagen I synthesis and metabolism, improving the formation of normal collagen fibrils and the production of a mineralized bone in an '*ex vivo*' assay in immunodepressed mice.

OI is a genetic disease caused by mutations in procollagen I genes, encoding for the two polypeptide chains of collagen I, the major protein constituent of bone extracellular matrix (ECM). It is characterized by reduced bone collagen content and mineralization, leading to brittle bones. The severe forms of OI are caused by mutations that, by altering the precise sequence of glycine residues in the triple helical region of collagen molecules, affect collagen folding, assembly and secretion in ECM. In these cases, one single chain with a mutation is sufficient to inactivate two normal associated chains, leading to a dominant-negative effect over the normal allele. Null mutations in one allele give very mild symptoms of OI, since the functional allele can supply enough collagen I protein to sustain a normal bone development, as in Mov13 heterozygous mice.

Up to now, gene therapy strategies have mainly addressed recessive diseases, which, affecting both alleles, lead to the reduction or the absence of a specific gene product. In these cases, the goal of the therapy is to supply the patient with a normal gene that can produce the missing protein. The viral vectors for gene therapy are engineered simply to deliver to the patient the curative dose of a missing gene product. This kind of approach is not feasible in those diseases, like OI, where the polypeptide produced by the mutated allele can inactivate the product of normal allele. In this case, the only possible strategy is to abolish the expression of the 'bad' allele.

In theory, there are two possible approaches for knocking out the expression of a dominant-negative allele. One is to achieve degradation of the mutant mRNA. Ribozyme or RNA interference can potentially be useful in this task. In one report, a hammerhead ribozyme was used to degrade selectively the mRNA of mutated OI allele.[3] However, with this strategy it was impossible to reach complete *COL1A1* mRNA inactivation, and, due to the high variety of different collagen mutations, ribozyme was directed toward a polymorphism of the mutated allele rather than the OI causative mutation. Therefore, the approach requires that the OI allele also carries the ribozyme-specific polymorphism and that the alleles would be distinguishable from each other.

The other approach, used by Chamberlain *et al*, is to disrupt the mutated allele by allele-specific viral insertion. The conversion of the dominant-negative allele to a null form leads to a much milder OI phenotype, since it can relieve the normal allele of the negative effect of the mutant collagen. The elevated targeting frequency that was observed in the *COL1A1* locus cannot distinguish between alleles; however, it is surprising to see improvement in collagen synthesis in the clones expressing only the mutant procollagen, or in a mixed polyclonal population. The production of a unique procollagen I type per individual cell (even of the mutant one) precluding the mixing of peptide chains is beneficial for osteoblasts derived from MSC and potentially useful for OI patients, as this simulates the mild clinical course of genetic mosaicism for lethal OI alleles.

Another remarkable point in Chamberlain's study is the use of gene disruption in adult stem cells. This evidence demonstrates that gene targeting is possible in nonembryonic human stem cells and opens new avenues in the therapy of a number of diseases. In the specific case of OI though, the beneficial effects of the reinfusion of modified MSC may be jeopardized by the poor ability of the MSC to extravasate, resulting in very inefficient engraftment.[4]

It is conceivable that this first example of gene therapy of a dominant-negative disease through an insertional knock out will be followed by numerous others. Knocking out the disease-causing allele is an attractive strategy for gene therapy of many dominant-negative disorders; however, two improvements will be necessary for a large-scale use of this approach. First, a tool needs to be developed to distinguish between the alleles and to inactivate selectively the disease-causing allele. The other definitive improvement of the strategy will be to achieve allele-specific gene inactivation *in vivo* by infection of patients with locus-specific AAVs. At that point the dream of gene therapy by direct gene replacement or correction may become a reality. Then we will enter the age of molecular surgery! ■

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