Patient-derived iPSCs reveals that genotype is a key factor to determine cellular phenotype and drug sensitivity in type II collagenopathy

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INTRODUCTION: Type II collagenopathy is a group of disorders caused by germline mutations in the COL2A1 gene. Before being unified under this category, many of these conditions had been classified as distinct diseases based on their clinical phenotypes, which range widely from neonatal lethality to early-onset osteoarthritis. Genomic studies of affected patients have suggested that this phenotypic diversity is largely determined by the type and location of the COL2A1 mutation. However, cases with identical mutations but markedly different clinical manifestations have also been reported, indicating that the influence of genetic background cannot be excluded. Here, we present our approach to addressing this issue using a series of gene-edited iPSCs generated from patient-derived iPSCs.

METHODS: The original iPSC line was established from a patient initially diagnosed with idiopathic osteonecrosis of the femoral head (INO). Subsequent genetic analysis revealed that the patient carried a point mutation in the *COL2A1* gene (Gly450Ala, hereafter referred to as ION-Ala). From a single clone of this patient, we generated a series of gene-edited iPSCs: one corrected to carry the normal allele (Gly450Gly, hereafter ION-Gly), one edited to harbor a mutant allele previously identified in a patient with hypochondrogenesis (Gly450Arg, hereafter ION-Arg), and another edited to carry a mutant allele found in a patient with achondrogenesis (Gly450Asp, hereafter ION-Asp). Chondrogenic induction of these cells was performed using our previously established somite-based differentiation method (reference 1). The properties of the resulting cells were assessed by evaluating cartilage-pellet formation and by analyzing the expression of cartilage-related genes through mRNA quantification and immunohistochemical staining. The induction of ER stress-related genes was also examined. Furthermore, the formation of growth plate-like structures and endochondral ossification were evaluated by transplanting the cartilage pellets into immunodeficient mice. As controls, iPSCs derived from an achondrogenesis patient (ACGII) and from a healthy donor (414C2) were included.

RESULTS: All gene-edited iPSC lines exhibited growth properties comparable to those of the original clone (ION-Ala). The expression of pluripotency marker genes and differentiation into the three germ layers were confirmed by in vitro embryoid body formation and teratoma generation in immunodeficient mice, indicating no significant phenotypic differences among the gene-edited iPSCs at the pluripotent stage. In contrast, their responses to chondrogenic induction varied considerably. At day 14 of induction, prior to the onset of *COL2A1* expression, no obvious morphological or immunohistochemical differences were observed among the clones. By day 28, however, ION-Ala and ION-Gly formed round, solid pellets similar to those of the normal control line 414C2, whereas ION-Arg and particularly ION-Asp failed to generate well-formed pellets, resembling the features observed in ACGII. Consistently, the expression of late chondrogenic markers, such as *COL10A1*, was reduced in parallel with these phenotypic differences. Expression of ER stress-related genes also varied among clones and correlated with the severity of the phenotypic abnormalities. Following transplantation, pellets harvested at day 28 were evaluated after 42 days. 414C2, ION-Gly, and ION-Ala showed robust endochondral ossification throughout the pellets, with clear COL10 expression in hypertrophic cartilage regions. In contrast, pellets derived from ION-Arg and ION-Asp exhibited little to no ossification. Taken together, these results demonstrate a clear association between genotype and phenotype in the chondrogenic differentiation response.
Because CHOP expression was strongly associated with the phenotypes observed in the mutant clones, we tested whether inhibition of CHOP could rescue the defects. Cells were treated with a CHOP inhibitor (CHOPi) from day 14 to day 28 of chondrogenic induction. CHOPi treatment improved pellet morphology and restored COL10A1 expression. Pellets collected at day 28 and transplanted into immunodeficient mice demonstrated growth plate–like structures with ossification after 42 days, indicating that chondrocytes rescued by CHOPi in vitro were also functional in vivo. These findings strongly support the genotype-phenotype correlation in this series of *COL2A1* mutations.

DISCUSSION: Recent advances in genome analysis of genetic skeletal disorders have led to the adoption of molecular classifications, with many diseases now grouped according to their causative genes. Consequently, it has become increasingly important to evaluate the biological effects of individual mutations in order to clarify their role in pathogenesis and to develop appropriate therapeutics. Patient-derived iPSCs provide an invaluable tool for this purpose. In this study, we demonstrated for the first time clear evidence that genotype is a key determinant of phenotype, at least at the cellular level. Our series of isogenic iPSC lines, sharing an identical genomic background, represent a powerful platform to investigate pathogenic mechanisms and to assess the effects of candidate therapeutic compounds. Of particular interest, short-term inhibition of ER stress–related pathways in vitro rescued the in vivo phenotype. Although the precise mechanism remains to be elucidated, these findings suggest that identifying a therapeutic time window could help minimize drug.

SIGNIFICANCE/CLINICAL RELEVANCE: Patient-derived iPSCs will be very useful materials to investigate the role of mutation and evaluate the effect of candidate therapeutic materials.

REFERENCES: 1. Pretemer Y, et al. Differentiation of Hypertrophic Chondrocytes from Human iPSCs for the In Vitro Modeling of Chondrodysplasias. Stem Cell Reports. 2021;16(3):610-25.

Figure 1

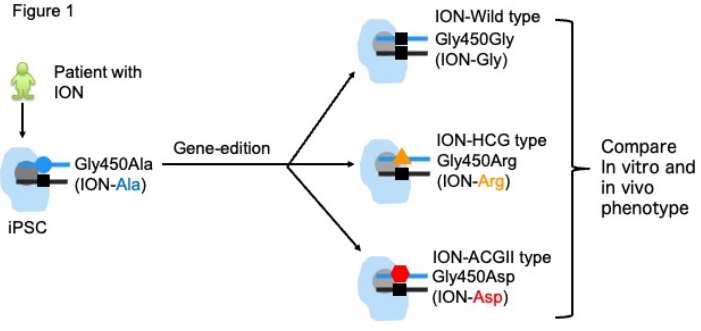

Figure 2. in vitro pellets stained by safranin-O

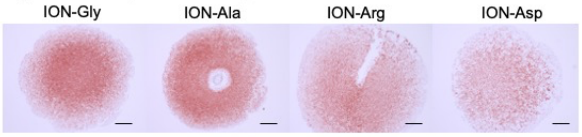

Figure 3. in vivo cartilage pellets treated w/o or w/ CHOPi stained with von Kossa