Addendum

Disease modeling of a mutation in α-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy

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Addendum to: EMBO Mol Med (2019) 11:e11115. DOI 10.15252/emmm.201911115 | Published online 3 November 2019

The authors recently contacted the journal to inform the editor of changes they discovered in the isogenic control (HCMrep) clone derived from the hypertrophic cardiomyopathy (HCM)-specific hiPSC clone. After the initial publication, the authors performed a new genomic sequencing of the HCM and HCMrep hiPSC clones, which revealed in the HCMrep an additional point mutation (T>C, blue in Figure 1).

Figure 1. Validation of the HCM and HCMrep hiPSC lines.
Sequencing of the HCM and HCMrep hiPSC clones. The HCMrep clone revealed on the +strand the repaired mutation (T>C (repair), violet), the 2 silent mutations from the repair template (C>A and G>C, red), and the on-target defect (T>C (on-target), blue). Note that the SnapGene software sometimes underlined the presence of a mutation with a red square at the nucleotide site, but sometimes did not.
Figure 2.
Fig 1) next to the CRISPR/Cas9-mediated repair of the HCM gene variant (T>C, violet). This heterozygous mutation (c.697+2T>C) is an on-target artifact of CRISPR/Cas9 gene editing and is located in the donor splice site of ACTN2 intron 8.

The authors evaluated whether this additional c.697+2T>C transition may affect the mRNA and protein pattern in cultured HCMrep hiPSC-derived cardiomyocytes (Fig 2). RT–PCR revealed additional bands after treatment with emetine, which blocks translation and prevents the degradation of nonsense mRNAs (Fig 2A). Sequencing of emetine-treated RT–PCR subclones revealed wild-type mRNA (Fig 2B) in 72% of cases and two nonsense mRNAs (Fig 2C and D) in 28% of cases. Both mutant mRNAs give rise to a frameshift and a premature termination codon, leading to C-terminal-truncated proteins. Western blot for ACTN2 revealed only one band without any truncated protein at the estimated molecular weight of about 30 kDa in HCMrep hiPSC-derived cardiomyocytes (Fig 2E).

These data showed that HCMrep hiPSC cardiomyocytes exhibit only wild-type ACTN2 mRNA in the absence of emetine and only the wild-type full-length ACTN2 protein. This addendum indicates that the original data are not affected by this newly discovered Cas9-mediated on-target mutation.