Tuning the Response of Synthetic Mechanogenetic Gene Circuits in Human iPSC-derived Chondrocytes using Mutations in the Mechanosensory Ion Channel TRPV4

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INTRODUCTION: The field of synthetic biology has opened new possibilities to develop biological tools to engineer cells for personal, precision medicine. A new area of synthetic biology – termed mechanogenetics – has successfully harnessed the power of mechanobiology to develop gene circuits that can be activated via various physiological stimuli such as mechanical loading or hypo-osmotic stimulation [1]. In particular, a subclass of mechanogenetic gene circuits based on activation of the mechanosensory ion channel transient receptor potential vanilloid-4 (TRPV4) has shown a great potential in being used as a cell-based therapeutic for osteoarthritis [2]. In this study, we hypothesized that the mechanogenetic circuit response could be tuned by directly modulating the sensitivity of TRPV4. To this effect, we developed mechanogenetic gene circuits in hiPSC-derived chondrocytes, genome-edited using CRISPR-Cas9 to harbor two specific mutants of TRPV4, V620I and T89I, which have been previously identified to show altered mechanobiologic and osmotically-sensitive responses [3][4]. The two mutations have been shown to increase the open probability of affected TRPV4 channels, which in turn raise the magnitude of both the basal current and Ca^{2+} influx. Our aim was to fabricate mechanoresponsive 3D engineered human cartilage constructs and to determine how mutation of the TRPV4 channel may be used to tune the gene circuit functions in human iPSC cell lines.

METHODS: Three hiPSC cell lines – two mutant lines, each containing V620I or T89I mutation in the *TRPV4* gene, and one isogenic control – underwent mesodermal lineage differentiation towards chondroprogenitors cells (Cps) [3][5]. Cps were further differentiated into chondrocytes using a high-density pellet culture protocol. After 42 days, mature chondrocytes were isolated from pellets using enzymatic digestion and were cryopreserved. For each batch of experiments, chondrocytes were thawed and passaged once before being lentivirally transduced with TRPV4-responsive mechanogenetic circuit containing a synthetic mechanosensitive NF-κB-inducible promoter upstream of a luciferase gene (NFKBr-Luc) [2][6]. The transduced cells were allowed to recover for two days, and used to fabricate 3D mechanogenetic cartilage constructs by encapsulating the cells into 2% (w/v) agarose at a density of 10 million cells/ml. To measure the circuit response, constructs were first cultured in an environmentally controlled plate reader for 24 hours at 37°C and 5% CO_2 to measure their baseline bioluminescent output. For pharmacological activation of TRPV4, constructs were stimulated with GSK101 or DMSO (control) for 15 minutes. For hypo-osmotic activation, constructs were cultured in either iso-osmotic or hypo-osmotic (135 mOsm) media for three hours. Constructs were washed with warm PBS before being placed back into the plate reader for 24 hours to obtain bioluminescence.

RESULTS: We first tested the mechanogenetic circuit response using GSK101, a pharmacological agonist of TRPV4. All three cell lines showed dose-dependent response profile, with mutants showing distinct trends (Fig. 1A-C). V620I mutant demonstrated significantly higher circuit activation at lower dosages (0.5 and 1 nM) (Fig. 2A), as well as higher peak response (Fig. 2B) compared to WT. T89I mutant showed an opposite response trend to that of V620I, where the mutant exhibited weaker circuit response compared to WT at GSK101 dosages higher than 1 nM (Fig. 2A,B). At higher dosages of GSK101, both mutants reached peak circuit response faster than WT (Fig. 2C). All mechanogenetic constructs were also capable of showing circuit response to hypo-osmotic loading, with strongest circuit response being observed from WT (Fig. 3A). Both mutants showed attenuated circuit response (Fig. 3B,C).

DISCUSSION: Here, we demonstrate for the first time the application of synthetic mechanogenetic circuits in human iPSC-derived chondrocytes, and further show that the circuit response can be modified by prescribed mutations in the TRPV4 channel. Both mutants showed distinct circuit response trends. The higher circuit response observed from V620I compared to WT at low GSK101 concentrations may indicate that the V620I mutation causes the TRPV4 channel to open more readily compared to WT (Fig. 2A). This result is also in line with previous studies that linked V620I and T89I mutations with increased open probability of TRPV4 [3]. However, the opposite trend was observed from the T89I mutant, which showed significantly lower circuit response than WT at GSK101 doses higher than 1 nM. Meanwhile, both mutants showed similar, weaker response to hypo-osmotic stimulation compared to WT (Fig. 3). These results suggest that the mutations may be playing disparate roles in how the mechanogenetic circuit responds to pharmacological versus physiological stimuli. Future work combining other mechanogenetic circuits, such as NFKBr-IL-1Ra, and more cell lines with different mutations in TRPV4 will provide us with a more clinically relevant data that could be used to understand how TRPV4 mutations will affect the output of therapeutics.

SIGNIFICANCE/CLINICAL RELEVANCE: Our approach can not only be adopted to test other mechanogenetic gene circuits, but also to tune their response by engineering the channel with site-directed mutagenesis. These studies will assist in developing personalized medicine by incorporating patient-specific iPSCs engineered to express a mutated variant of TRPV4 that allow for tunable responses to physiological stimuli.

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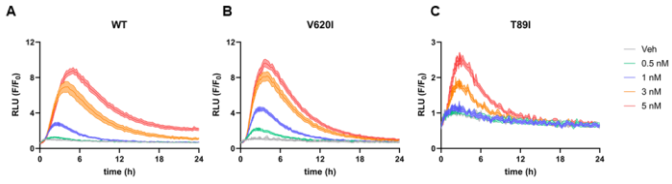

Figure 1. Normalized luminescence curves of NFKBr-Luc-transduced WT (A), V620I (B), and T89I (C) constructs in response to GSK101 stimulation. Data represented as Mean ± SEM (n = 6 per group). RLU: relative luminescence units

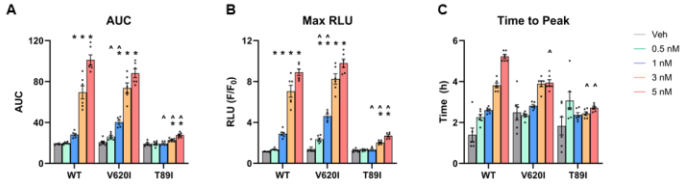

Figure 2. Quantitative analyses of circuit response (Fig. 1A-C). Data represented as Mean ± SEM (n = 6 per group). AUC, area under the curve. * denote significant difference from vehicle group (within each cell line, $p < 0.05$). ^ denote significant difference from WT (within each condition, $p < 0.05$).

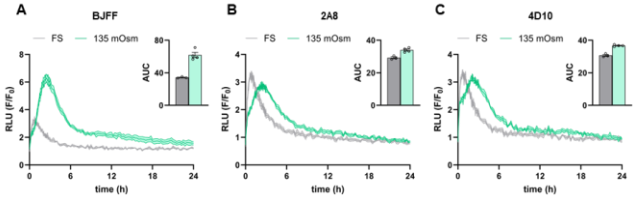

Figure 3. Normalized luminescence curves of NFKBr-Luc-transduced WT (A), V620I (B), and T89I (C) constructs in response to iso-osmotic (FS, Free Swelling) or hypo-osmotic stimulation. Data represented as Mean ± SEM (n = 3 per group).