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Transient and local modulation of cardiomyocyte cell function by intracellular protein delivery in vivo

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Purpose: During an ischemic insult part of the heart is exposed to hypoxic and acidic conditions resulting in the loss of a vast number of cardiomyocytes. The heart lacks the regenerative capacity to replenish the cardiomyocyte pool and therefore a major therapeutic goal is to promote cardiomyocyte proliferation and cytokinesis. Recently, a novel methodology termed induced transduction by osmocytosis and propanebetaine (iTOP) was described that allows transient intracellular delivery of recombinant proteins in vitro; however, delivery of proteins in vivo has not been demonstrated. Here, we aimed to apply iTOP for the delivery of recombinant proteins to the heart under physiological and ischemic conditions.

Methods: To investigate whether iTOP is suitable for delivery of proteins to the heart we injected recombinant Cre into the left ventricular wall of a Rosa26mT/mG mice using two 15µl injections and harvested tissues two weeks later. Upon transduction, Cre-mediated excision of the tdTomato-STOP genetic locus occurs resulting in the subsequent expression of eGFP. We used the traceability of this model to study transduction efficiency of recombinant protein and assessed whether the buffer osmolarity influenced the degree of transduction by modifying the NaCl concentration (500, 800, 1250, 1800, 2500 mOsm). Furthermore, based on stereomicroscopy and confocal imaging we assessed off-target recombination in the liver, kidney, lung and spleen. Next, we subjected mice to an ischemia-reperfusion injury followed by an intra-cardiac injection of recombinant Cre protein and iTOP buffer with the aim to study the transduction dynamics of recombinant protein under hypoxic conditions. Finally, to assess whether transient modulation of cell function can promote cardiac regeneration, we generated a constitutively active traceable and non-traceable variant of yes associated protein 1 (Cre-YAP5SA and YAP5SA) which is known to promote cardiomyocyte regeneration.

Results: Stereomicroscope and confocal imaging showed efficient Cre-mediated recombination near the intra-cardiac injection sites which was independent of the buffer osmolarity. Importantly, no Cre-mediated recombination was observed in the liver, kidney, lung, and spleen. Furthermore, cardiac ischemia did not influence the transduction efficiency of recombinant Cre protein via iTOP. Finally, activity assays determined the biological activity of Cre-YAP5SA and YAP5SA in vitro. Conclusions: Utilizing a novel methodology, we show efficient and local delivery of recombinant protein to the heart both under physiological and ischemic conditions. Future studies will focus on the delivery of the transcriptional regulator YAP5SA with the aim to promote the proliferative capacity of cardiomyocytes and augment cardiac regeneration after an ischemic insult.