Safia Costes, Damien Laouteouet, Magalie Ravier, Morgane Delobel, Gyslaine Bertrand, Stephane Dalle, Aleksey V. Matveyenko

Circadian clock nuclear receptor REV-ERBα is a novel regulator of beta-cell function, survival and autophagy under diabetogenic conditions

Background and aims:
The circadian clock regulates diverse cellular and molecular rhythms employing CLOCK-BMAL1 transcriptional heterodimer with nuclear receptor REV-ERBα (encoded by gene *Nr1d1*) playing an important role as a clock repressor through modulation of *Bmal1* transcription. Importantly, in addition to its core circadian clock function, recent studies have identified REV-ERBα as a potent transcriptional repressor of autophagy. Therefore, in the current study we set out to address whether impaired beta-cell function and survival associated with exposure to diabetogenic stressors (e.g. glucotoxicty and inflammation) is attributed in part to REV-ERBα-mediated inhibition of autophagy.

Materials and methods: Experiments were performed with the rat pancreatic beta-cell line (INS-1E). p62 (also known as sequestosome-1) levels were used to monitor autophagic degradation and evaluated by western blot. Because p62 aggregated forms were reported to be largely insoluble, we also evaluated the detergent-solubility of p62 by fractionation and western blot analysis. Apoptosis was evidenced by cleaved caspase-3 emergence. Glucose-induced insulin secretion was assessed by Homogeneous Time Resolved Fluorescence (HTRF) technology.

Results:
Exposure of beta-cells to either glucotoxicity (30 mM glucose for 48h) or cytokines (cytomix of IL-1β, TNFα and IFNγ for 24h) resulted in robust induction of REV-ERBα expression (1.5-2 fold, $p<0.05$) and corresponded with impaired autophagy flux characterized by increased protein levels of p62 (1.5-2 fold, $p<0.05$). Consistent with these data, exposure of beta-cells to a REV-ERBα agonist (SR9011) was characterized by impaired autophagy (increased p62 levels, aggregated and insoluble forms, $p<0.05$), defective glucose-stimulated insulin secretion (70 % decrease, $p<0.05$) and increased beta-cell apoptosis (increased cleaved caspase-3, $p<0.01$ vs. vehicle). In contrast, REV-ERBα specific antagonist (SR8278) protected beta-cells from deleterious effects of glucotoxicity or cytokines-induced inflammation by enhancing autophagy flux and attenuating beta-cell apoptosis (~30%).

Conclusion:
Taken together, these data reveal for the first time an underexplored link between the core circadian clock nuclear receptor REV-ERBα, autophagy and beta-cell failure under diabetogenic conditions. These data also suggest a potential therapeutic potential of modulating REV-ERBα levels in beta-cells to enhance function and survival in diabetes.