FUNCTIONAL CHARACTERIZATION OF A XENOPUS TROPICALIS KNOCKOUT AND A HUMAN CELLULAR MODEL OF RCBTB1-ASSOCIATED INHERITED RETINAL DISEASE SHOWS INVOLVEMENT OF RCBTB1 IN THE CELLULAR RESPONSE TO OXIDATIVE STRESS

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The function of RCBTB1, implicated in syndromic and non-syndromic inherited retinal disease (IRD), remains unknown so far. Patients with biallelic missense variants in RCBTB1 display diverse IRD phenotypes such as retinitis pigmentosa. Here, we tested the hypothesis that RCBTB1 is involved in NRF2-regulated protection against oxidative stress in the eye, more specifically in the retinal pigment epithelium (RPE). A Xenopus tropicalis knockout (KO) was generated using CRISPR/Cas9 editing. Histological examination and three-dimensional electron microscopy was performed on retinas of rcbtb1-/- frogs. RNA- seq analysis was performed on RCBTB1-mutated patients' lymphocytes, treated with H2O2, as well as on embryos from the rcbtb1-/- KOs treated with CdCl2. A knockdown (KD) cell line was generated in ARPE-19 cells and functional assays (flow cytometry, MTT- assay, cell death kinetics) assessed the consequences of RCBTB1 loss-off function. The rcbtb1-/- animals showed changes in the RPE, similar to observations in human cases, including loss of apical-basal cell polarity, loss of cuboidal cell morphology, spreading of the pigment granules and vacuolisation. NRF2 downstream targets and several metallothioneins were found to be differentially expressed, both in the KO and cellular models. Functional assays in ARPE-19 cells revealed that RCBTB1 depletion affects cellular responses to external insults of oxidative stress. We showed that the Xenopus tropicalis rcbtb1-/- recapitulates the human IRD phenotype. Both in vivo and in vitro functional data show involvement of RCBTB1 in the cellular response to oxidative stress. This provides insight into the mechanism underlying RCBTB1-associated IRD and uncovers potential therapeutic opportunities.