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Chemical hypoxia-induced integrated stress response activation in oligodendrocytes is mediated by the transcription factor nuclear factor (erythroid-derived 2)-like 2 (NRF2)

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Introduction

The extent of remyelination in multiple sclerosis (MS) lesions is often incomplete (Patrikios *et al.* 2006). Oligodendrocyte progenitor cell (OPC) death can be a contributing factor for such incomplete remyelination (Dincman *et al.* 2016, Maus *et al.* 2015, Moore *et al.* 2015). The precise mechanisms underlying insufficient repair remain to be defined, but oxidative stress appears to be involved. Several studies support the view that oxidative stress has a strong connection with the integrated stress response [ISR; reviewed in (Cao & Kaufman 2014, Santos et al. 2009)]. To what extent such an oxidative-ISR crosstalk exists in oligodendrocytes is not well understood. Here, we used in vivo and in vitro approaches to investigate a causal relation of oxidative stress and endoplasmic reticulum stress signaling cascades.

Results

Chemical hypoxia induces the expression of key integrated stress response transcription factors

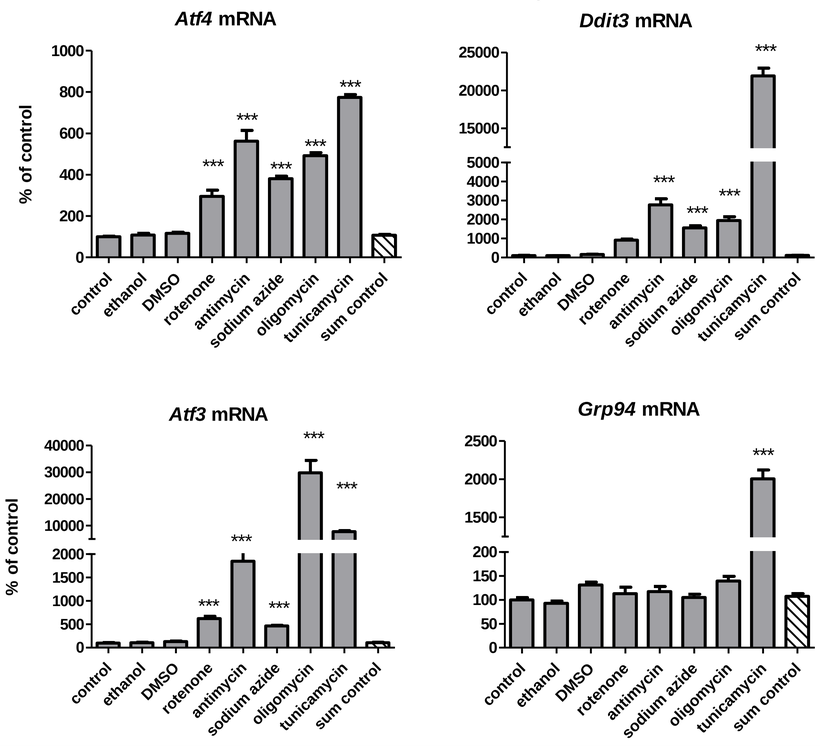

Figure 1: *Atf3*, *Atf4*, *Ddit3* and *Grp94* mRNA expression in OLN93 cells, stressed with different respiratory chain inhibitors and the N-linked glycosylation inhibitor tunicamycin. The following concentrations were used: rotenone (1µM), antimycin (10µM), sodium azide (10mM), oligomycin (10µM), tunicamycin (50µg/ml). Significant differences with respect to control cultures are indicated by *** p<0.001. In contrast to *Atf3*, *Atf4* and *Ddit3*, inhibition of the respiratory chain did not regulate the expression of the heat shock protein *Grp94*.

Sodium azide treatment induces loss of mitochondrial membrane potential

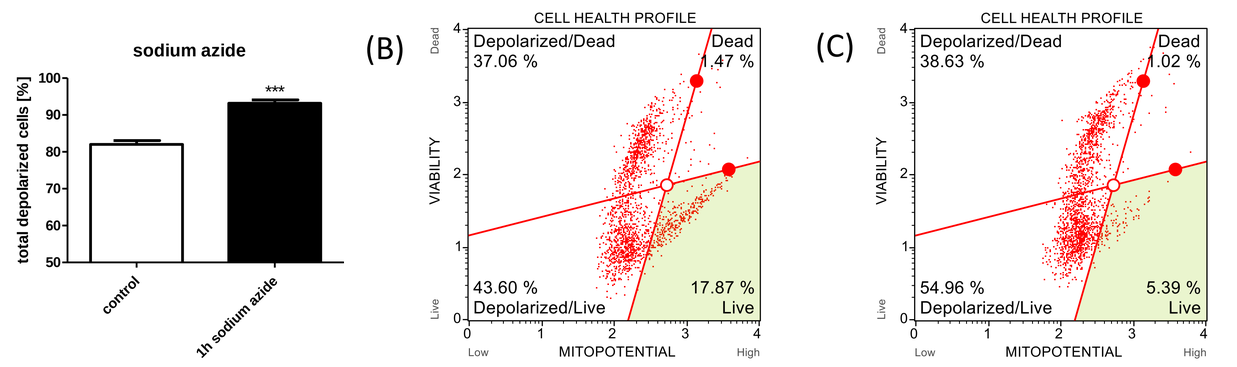

Figure 2: As shown in (A), numbers of depolarized cells after 1h sodium azide treatment were significantly higher compared to control cultures. Representative plots [control (B); sodium azide (C)] show the gated cells with four distinct cell populations: dead cells with a putative unspecific intact mitochondrial membrane potential (upper right); dead cells with a breakdown of the mitochondrial membrane potential (upper left); viable cells with an intact mitochondrial membrane potential (lower right); viable cells with a breakdown of the mitochondrial membrane potential (lower left). Significant differences with respect to control cultures are indicated by *** p<0.001.

Chemical hypoxia induces oxidative stress in OLN93 cells

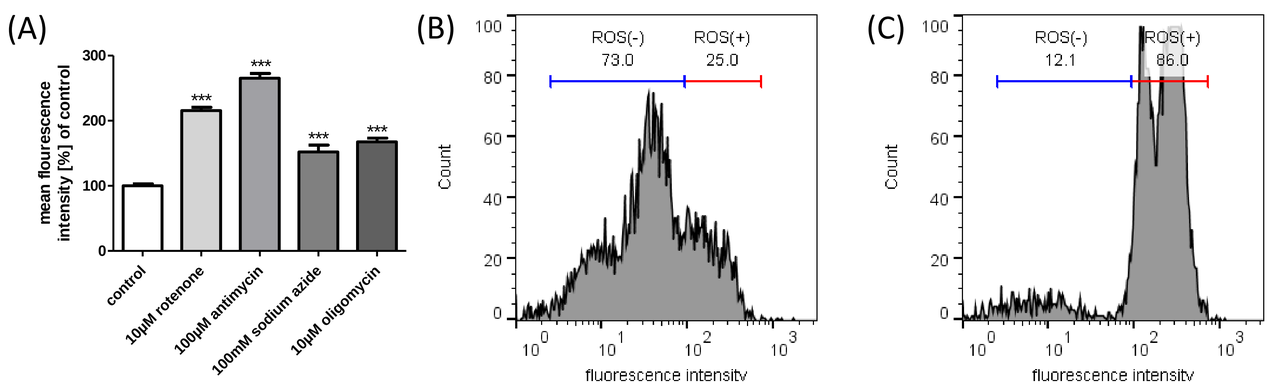

Figure 3: (A) Mean fluorescence intensity after dihydroethidium staining in control and stressed oligodendrocytes (1h), determined by flow cytometry analysis. Representative plots [control (B); antimycin (C)] show the histograms of gated cells with two markers providing data on two cell populations: reactive oxygen species negative and positive (ROS- and ROS+) cells. Significant differences with respect to control cultures are indicated by *** p<0.001.

Oligodendrocyte progenitor cells express DDIT3 and ATF3 in vitro & in vivo

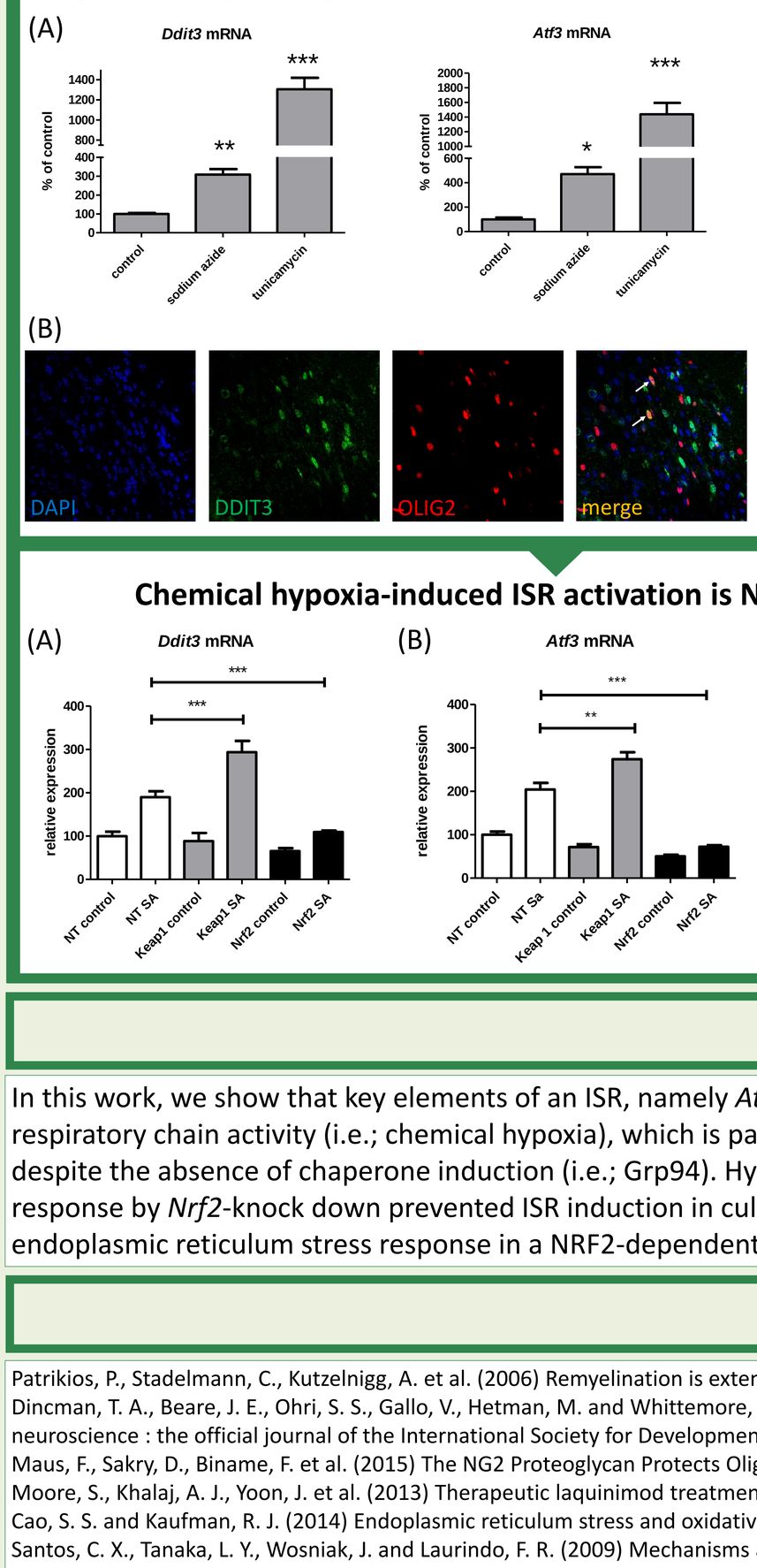

Figure 4: (A) Ddit3 and Atf3 mRNA expression in primary rat oligodendrocyte cultures stressed with the respiratory chain inhibitor sodium azide (10mM) or the N-linked glycosylation inhibitor tunicamycin (25µg/ml). Significant differences with respect to control cultures are indicated by * p<0.05, ** p<0.01, or *** p<0.001.
(B) Immunoflourescence-double-labelling to demonstrate DDIT3 expression in OLIG2+ oligodendrocyte progenitor cells in an in vivo remyelination model. For more information see Poster No. 25.

Chemical hypoxia-induced ISR activation is NRF2-dependant

Figure 5*:* Ddit3 and Atf3 mRNA expression levels after treatment with the complex IV inhibitor sodium azide (24h; 1mM) in the murine oligodendrocyte cell line OliNeu, in which Nrf2 or Keap1 gene expression was silenced by lentiviral shRNA delivery. Significant differences with respect to control cultures are indicated by ** p<0.01, or *** p<0.001.

Chemical hypoxia – mode of action

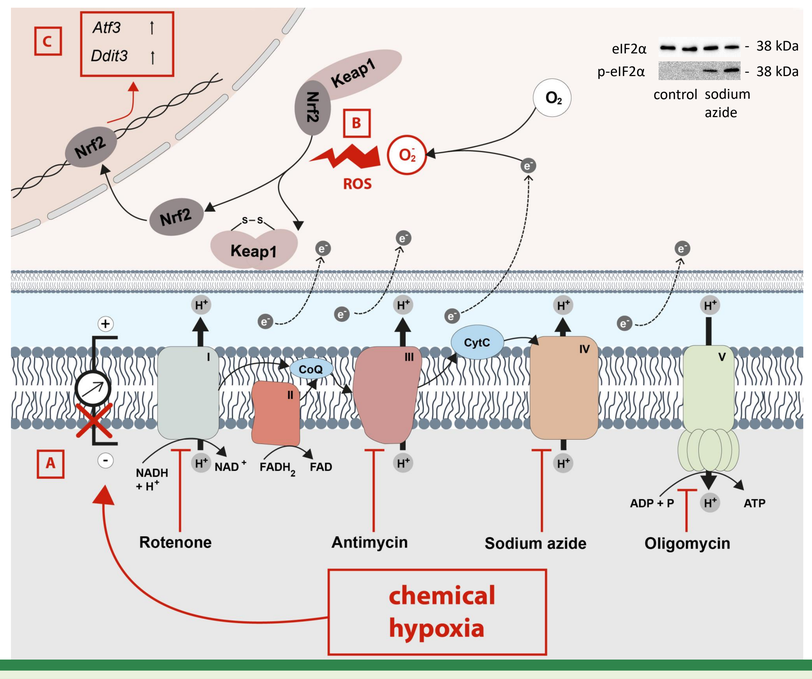

Figure 6: Chemical hypoxia induces loss of mitochondrial membrane potential (ΔΨm) (A). "Leaky" electrons can escape from the respiratory chain and reduce O_2, resulting in the generation of superoxide, the primary ROS (B). As a consequence, NRF2 is released from KEAP1 and translocates into the nucleus where it binds to the antioxidant response element (ARE), thereby activating the transcription of *Atf3* and *Ddit3* (C).

Discussion

In this work, we show that key elements of an ISR, namely *Atf3*, *Atf4* and *Ddit3,* are induced in OPCs through experimental ISR stimulation, but as well through inhibition of respiratory chain activity (i.e.; chemical hypoxia), which is paralleled by ROS accumulation. Induction of *Atf3*, *Atf4* and *Ddit3* expression by mitochondrial chain inhibitors occurred despite the absence of chaperone induction (i.e.; Grp94). Hyper-activation of NRF2-signaling by *Keap1*-knock down boosted ISR induction, whereas blocking of the oxidative stress response by *Nrf2*-knock down prevented ISR induction in cultured oligodendrocytes. This study provides strong evidence that oxidative stress in oligodendrocytes activates endoplasmic reticulum stress response in a NRF2-dependent manner and, in consequence, might regulate oligodendrocyte degeneration in MS and other neurological disorders.

Literature

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The authors declar no conflict of interest.