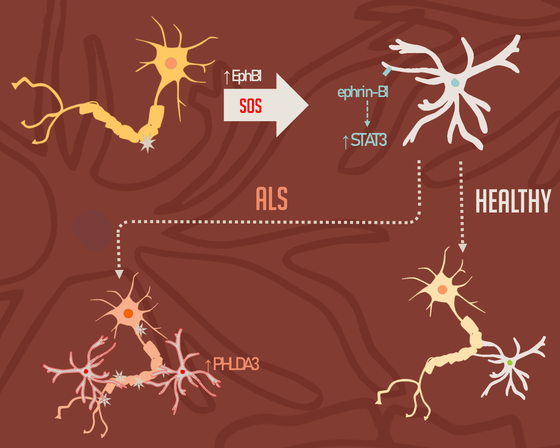
PHLDA3 OVEREXPRESSION IN ASTROCYTES CAUSES ENDOPLASMIC RETICULUM STRESS

I. Vargová[1,2], M. Řehořová[1,2], J. Turečková[1], J. C. F. Kwok[1,3], J. W. Fawcett[1,4], P. Jendelová[1,2]

[1]Institute of Experimental Medicine, Czech Academy of Science, Videnska 1083, 142 20 Prague, Czech Republic
[2]Second Faculty of Medicine, Charles University, V Uvalu 84, 150 06 Prague 5, Czech Republic
[3]Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom
[4]John van Geest Centre for Brain Repair, University of Cambridge, Cambridge CB2 0PY, United Kingdom

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by gradual loss of voluntary movement, caused by death of motor neurons (MNs). Although pathogenesis of ALS is unknown, previous studies have shown that astrocytes (ACs) from ALS patients have altered response to neuronal injury and contribute to death of MNs.



Recent evidence suggests that harmful, instead of favourable function of ACs in ALS could be caused by upregulation of pleckstrin homology-like domain, family A, member-3 (PHLDA3). PHLDA3 was recently discovered as a molecule participating in p53-dependent signalling and is activated in endoplasmic reticulum stress (ER stress). ER stress is one of the mechanisms implicated in the pathogenesis of ALS. The aim of this study is to investigate the role of PHLDA3 in the MN interaction with ACs during activation of ER stress in *in vitro* model.

METHODS

Primary ACs were isolated from the cortexes of 1-3 days old wild-type mice pups (P1-3). Culture purity was determined by immunocytochemistry to be 80% GLAST positive cells.

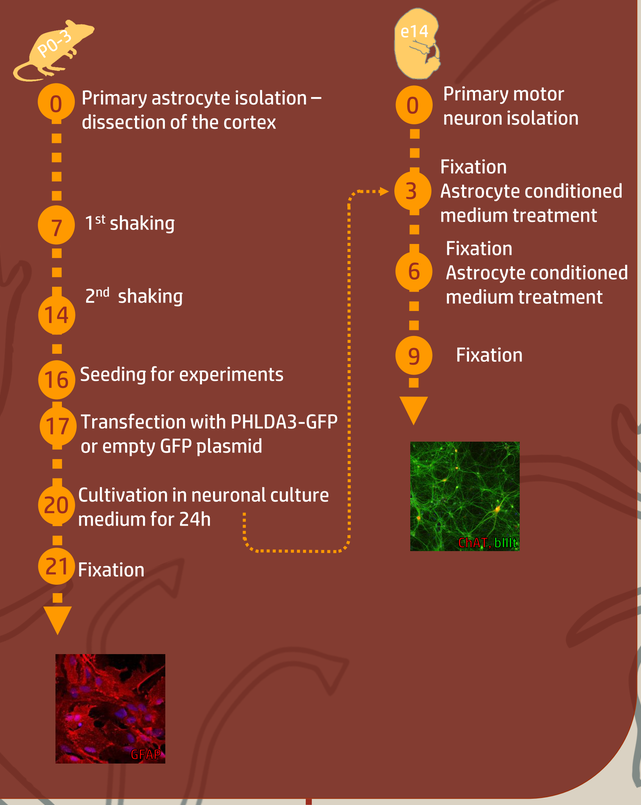
Primary MNs were isolated from wild-type mouse embryonic spinal cord (E14). Culture purity was determined by immunocytochemistry to be 65% βIII tubulin positive cells.

Upregulation of PHLDA3 in primary ACs was achieved by lipofectamine transfection with a plasmid containing PHLDA3 gene .

Western blot analysis was used to determine ER stress in ACs.

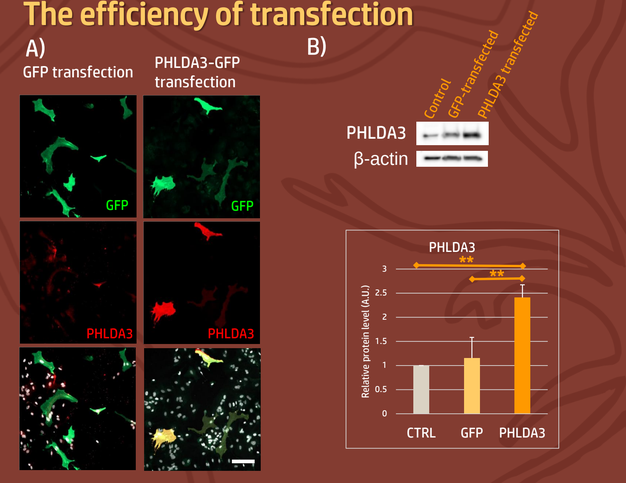
Effect of transfected ACs on MNs was determined by treating MN cultures with astrocyte-conditioned medium.

Experimental design



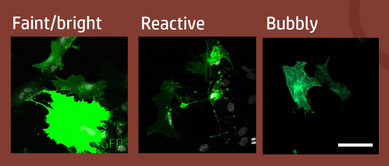
RESULTS

The efficiency of transfection



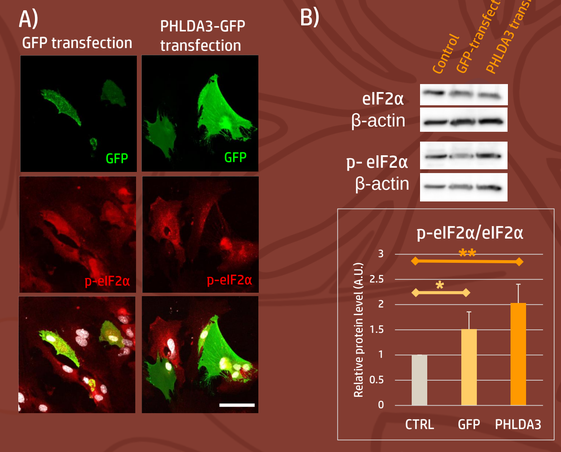
The verification of efficiency of PHLDA3-GFP plasmid delivery to primary mice astrocytes.
(A) Representative images of colocalization of anti-PHLDA3 (red) with transfected astrocytes area stained with anti-GFP (green). Scale bar: 50μm.
(B) Western blot comparison PHLDA3 level in astrocytes.

Effect of transfection on cell morphology



The transfection of the astrocytes with GFP plasmid or PHLDA3-GFP plasmid for overnight caused substantial morphological changes as some cells were reactive and some cells were with ”bubbly” shape suggesting cellular damage, however minimal morphological changes were observed in comparison with GFP and PHLDA3 transfection.

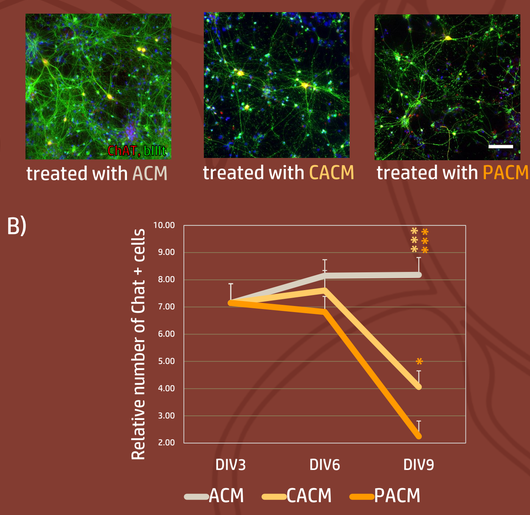
PHLDA3 expression in astrocytes increases level of p-eIF2α



Phosphorylation of eIF2α occurs by multiple stress-activated eIF2α kinases localized in the cytosol or on the membrane of endoplasmic reticulum. Several lines of evidence indicate that eIF2α phosphorylation stimulates activation of caspase -3 like protein and cell death.

(A) Representative images of immunofluorescent co-staining with anti-GFP (green) and p-eIF2α antibodies (red) in primary astrocytes transfected with PHLDA3-GFP or GFP control plasmid. Scale bar: 50μm.
(B) Western blot analysis shows elevated eIF2α phosphorylation level (p-eIF2α) in PHLDA3 transfected astrocytes.

Conditioned medium from PHLDA3 astrocytes decreases survival of motor neurons



(A) Representative images of neuronal cultures at day 9 of cultivation (DIV9), after treatment with astrocyte-conditioned media. Cells positive for neuronal marker βIII-tubulin (green) and ChAT (red) were regarded as surviving motor neurons. Scale bar: 100μm.
(B) Lowest motor neuron survival until DIV9 was observed in cultures treated with PHLDA3-transfected astrocyte conditioned medium (PACM) compared to control plasmid transfected astrocyte (CACM) and non-transfected astrocyte conditioned medium (ACM).

CONCLUSIONS

PHLDA3 overexpression increases level of p-eIF2α (ER stress marker) in astrocytes.

Medium conditioned with astrocytes overexpressing PHLDA3 has negative effect on survival of motor neurons.

PHLDA3-mediated ER stress in astrocytes is a potential mechanism contributing to pathophysiology of amyotrophic lateral sclerosis.

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