Insights into potential production of PERK protein isoforms resulting from EIF2AK3 alternative splicing

JoAnna James[1*,2], Elena Alvarez-Periel[2], Avinash Singh[1**,2], Kelly L. Jordan-Sciutto[2]

[1]College of Arts and Sciences, University of Pennsylvania (*C'2026,**C'2023); [2]Department of Oral Medicine, School of Dental Medicine, Philadelphia, Pennsylvania, USA

Introduction

PERK (protein kinase R-like ER kinase)

- One of the four kinases belonging to the integrated stress response (ISR) pathway
- In response to ER stress, it phosphorylates eIF2α, causing inhibition of cap-dependent protein translation[1]

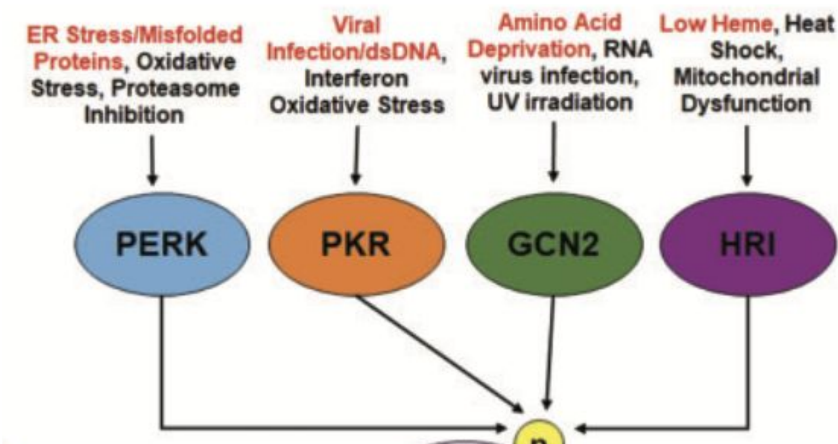

Figure 1. ISR signaling pathway[1]

Alternative splicing

- Can generate different mRNA isoforms which might result in alternative protein isoforms or mRNA transcripts with premature stop codons (PTCs)[2]
- The Ensembl database predicts several transcripts isoforms for *EIF2AK3*[3]

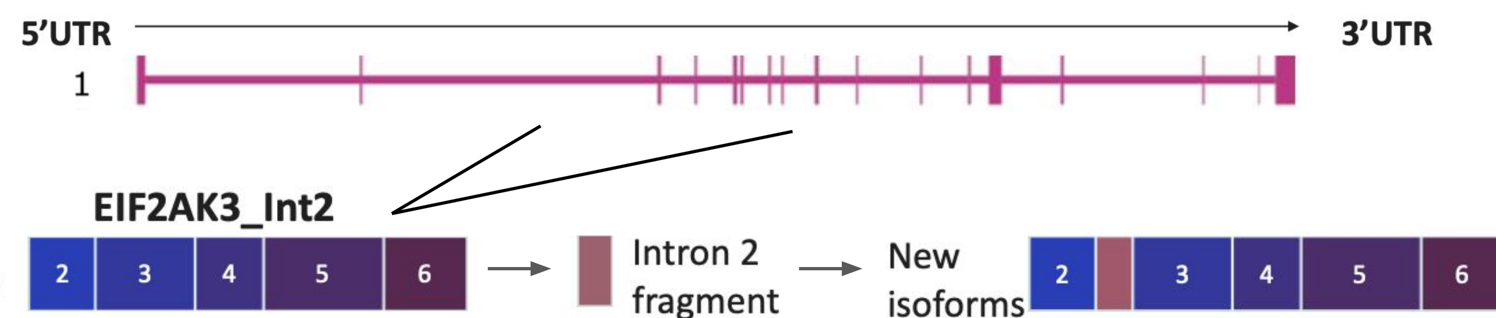

Figure 2. Example of an *EIF2AK3* alternative splicing transcript

Protein isoform

- Some of the identified transcripts are predicted to produce PERK isoforms[4]
- Preliminary western blot (WB) results detect multiple PERK bands

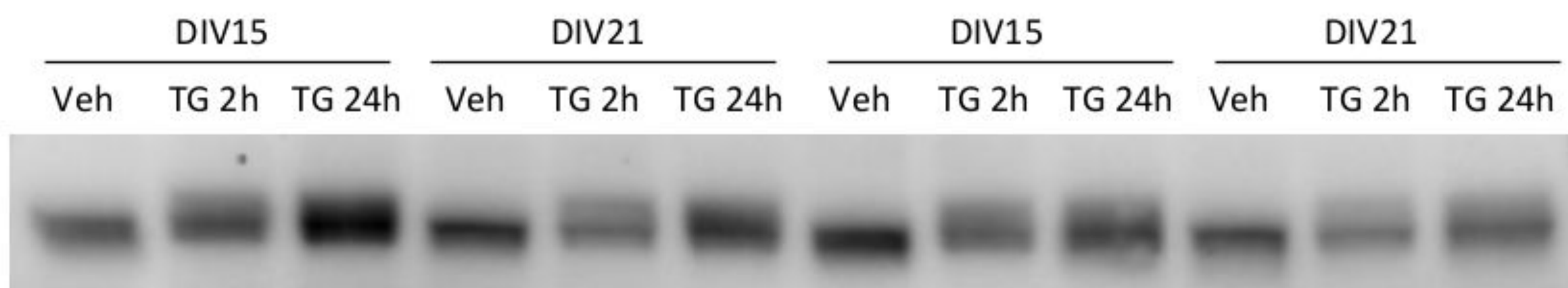

Figure 3. Neuronal cultures were grown in vitro for 15 or 21 days (DIV 15 and DIV 21), treated with DMSO (vehicle) or thapsigargin (TG), lysated, then ran on a WB. There appears to be an additional upper PERK band.

Results

1. Phosphatase treatment does not completely eliminate PERK shift

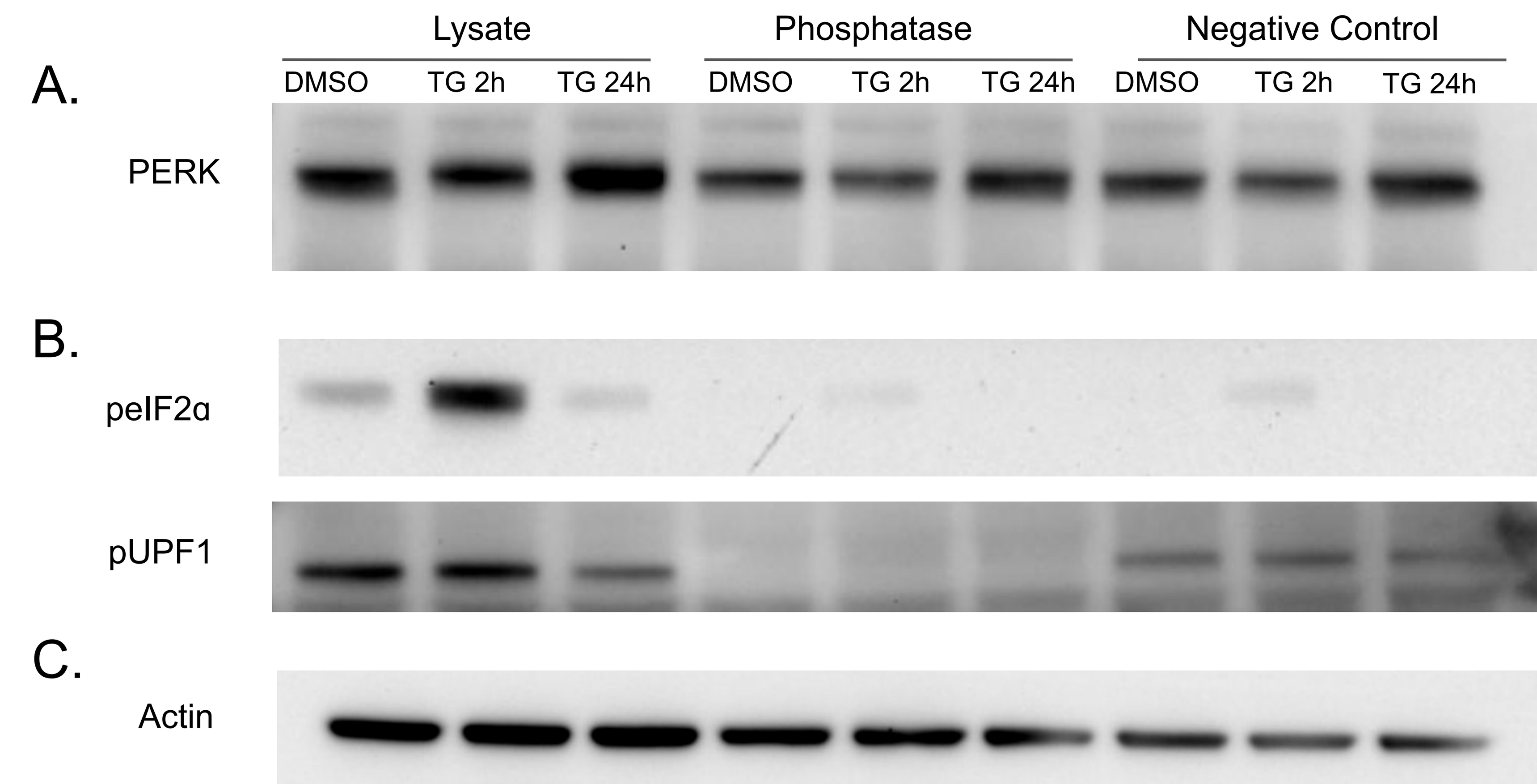

Figure 4. Neuronal cultures DIV (days in vitro) 21 are treated with DMSO or thapsigargin. Lysates were obtained from cultures and subdivided in three groups: one with no additional treatment (Lysate), one treated with CIP buffer and enzyme (Phosphatase), and one treated with only the CIP buffer (Negative Control). The antibodies for B are specific to the phosphorylated species. peIF2α and pUPF1 (B) serve as the control, and actin (C) is the loading control. Based on B, the CIP treatment appears to have worked.

2. Main PERK bands are specific

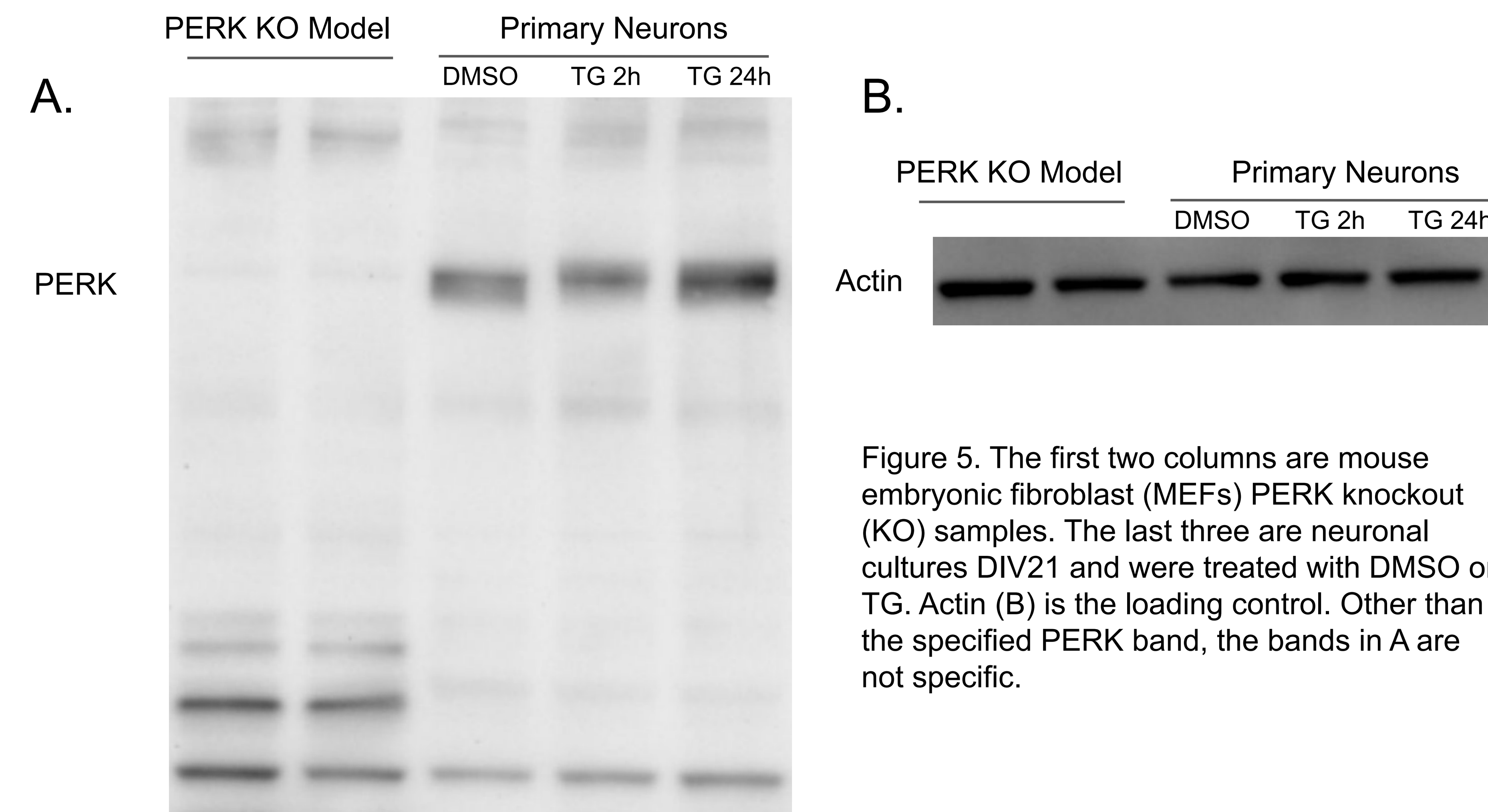

Figure 5. The first two columns are mouse embryonic fibroblast (MEFs) PERK knockout (KO) samples. The last three are neuronal cultures DIV21 and were treated with DMSO or TG. Actin (B) is the loading control. Other than the specified PERK band, the bands in A are not specific.

Aim

- To rule out whether additional bands observed via western blot for PERK are due to phosphorylation or might be non-specific bands

Methods

1. Primary mouse cortical neuronal cultures (day in vitro 21)

   ↓ DMSO + Thapsigargin (TG) treatment (150 nM for 2h or 24h)

   ↓ Lysate

   ↓ Calf intestinal alkaline phosphatase (CIP) treatment (none, CIP buffer, CIP buffer + enzyme)

   ↓ Western blot

2. Mouse embryonic fibroblasts (MEFs) PERK KO

   ↓ Lysate

   ↓ Western blot

Discussion

- The results support the idea that this PERK shift observed via WB is not caused by the presence of phosphorylation or due to antibody unspecificity, and thus, they might be the result of alternative PERK protein isoform.
- This study was the first step of a bigger project determining if alternative RNA spliced variants of PERK might result in different protein isoforms.
- Future studies better separating the WB bands are required to corroboration these results. Ultimately, proper identification of alternative protein isoforms will requires mass spectrometry studies.

References:

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