Pemetrexed Hinders Translation Inhibition upon Low Glucose in Non-Small Cell Lung Cancer Cells

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INTRODUCTION

Pemetrexed (PEM) is one of the most-extensively prescribed antifolate chemotherapeutic drugs for maintenance therapy of patients with locally advanced or metastatic Non-Small Cell Lung Cancer (NSCLC). This agent impedes the synthesis of folate and purine, which is crucial for the generation of nucleotides pool and cell replication. In addition, this impairment of purine production by PEM leads to ATP depletion that might impact numerous metabolic pathways, including protein synthesis that represents the most consuming energy cellular pathway.
Furthermore, poorly vascularized regions of solid tumors and the exacerbated metabolism of tumor cells can disrupt the nutritional homeostasis within tumoral microenvironment, leading to decreased concentrations of several nutrients including glucose. Tumor cell adaptation will thus depend on its capacity to orchestrate a molecular and metabolic program to cope with such metabolic stress. One major key in this process relies on the activation of the unfolded protein response (UPR) and particularly the eIF2a phosphorylation that provokes a cytoprotective repression of protein synthesis and cell cycle arrest. Chemically-induced phosphorylation of eIF2a protects tumor cells from antifolate treatment suggesting that this factor integrates nutritional and therapeutic stress signals. However, whether protein synthesis may be differently modulated by PEM when eIF2a is phosphorylated remains unknown.
In this study, we thus investigated whether translation and the UPR is differently affected by pemetrexed and glucose availability.

1 Experimental settings to assess impact of pemetrexed treatment on biomass independently of cytotoxicity

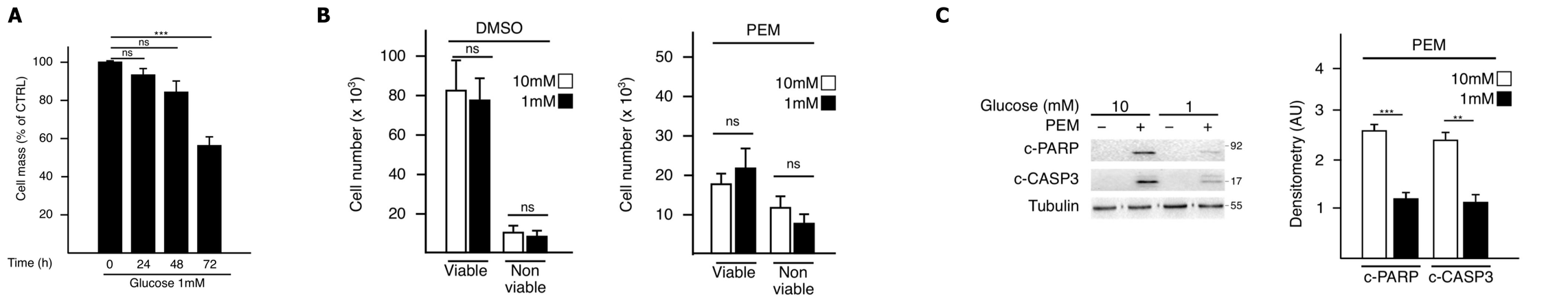

Figure 1
(A) Time course analysis of cell mass monitored by sulforhodamine B (SRB) assay performed on A549 cells cultured in 1 mM for the indicated time points. Control (CTRL) is referred to as the time point 0 h of deprivation. Data represent the mean of four independent experiments SEM.
(B) Cell viability was assessed using trypan blue exclusion assay after 72h of culture in medium containing 10 mM or 1 mM glucose and treated with DMSO or PEM (4 μM).
(C) Western blot analysis of apoptotic markers: cleaved forms of caspase 3 (c-CASP3) and PARP (c-PARP). For Western blotting, the most representative result from three independent experiments is displayed. Quantitative analysis was performed by comparing signals obtained in PEM-treated conditions normalized to tubulin using ImageJ software. ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

2 Pemetrexed determines the degree of translation impairment by low glucose

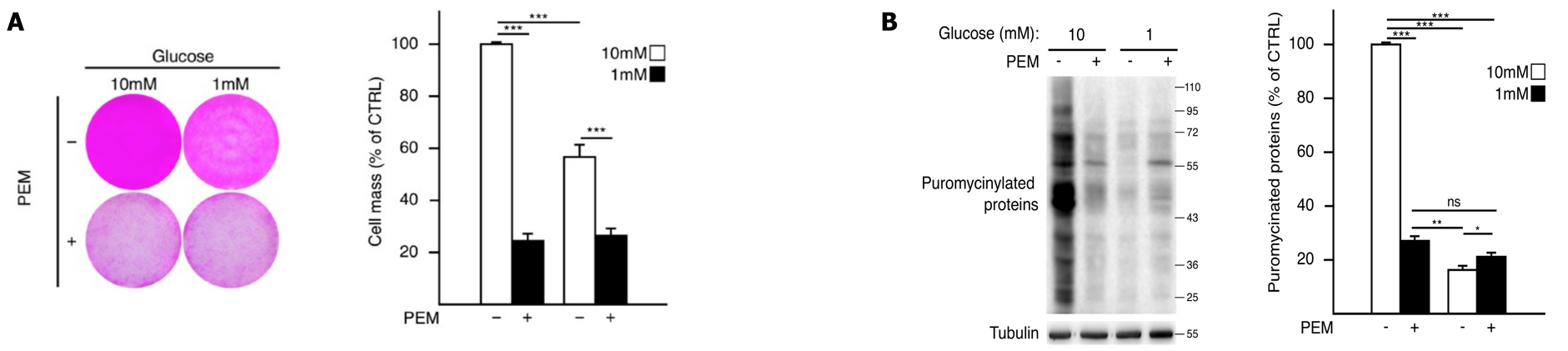

Figure 2
(A)After 72 h, in 10 mM or 1 mM glucose-containing medium, A549 cells were treated or not with PEM (4 μM), and protein mass was measured using a SRB assay. SRB staining was imaged before measuring the corresponding absorbance. Control (CTRL) is referred to as the 10 mM without PEM condition. Data represent the mean SEM (n = 8).
(B)The protein synthesis rate was assessed by SUnSET assay and quantified by measuring the ratio of puromycinilated proteins normalized against tubulin using the ImageJ software. Quantification data represent the mean SEM (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

3 Pemetrexed constrains the unfolded protein response (UPR)

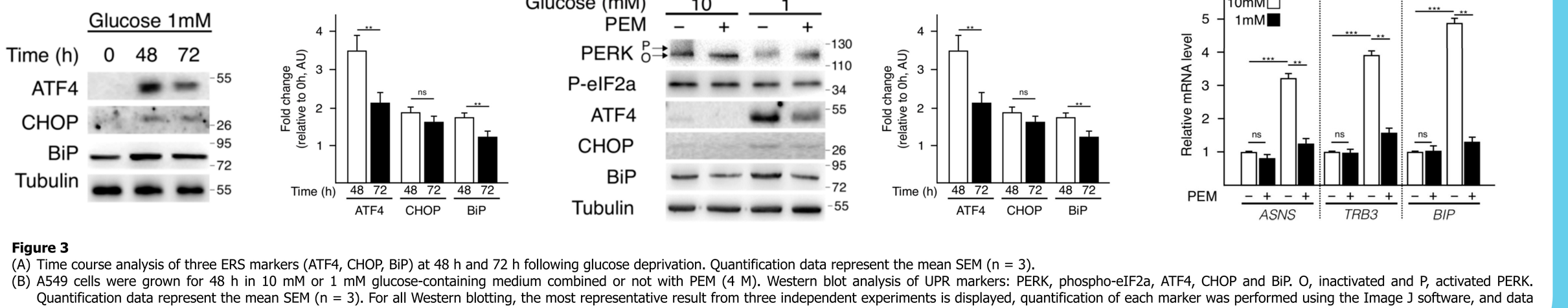

Figure 3
(A) Time course analysis of three ERS markers (ATF4, CHOP, BiP) at 48 h and 72 h following glucose deprivation. Quantification data represent the mean SEM (n = 3).
(B) A549 cells were grown for 48 h in 10 mM or 1 mM glucose-containing medium combined or not with PEM (4 M). Western blot analysis of UPR markers: PERK, phospho-eIF2a, ATF4, CHOP and BiP. O, inactivated and P, activated PERK. Quantification data represent the mean SEM (n = 3). For all Western blotting, the most representative result from three independent experiments is displayed, quantification of each marker was performed using the Image J software, and data are represented as fold change relative to the indicated condition.
(C) Expression measurements of canonical UPR-target genes ASNS, TRB3, and BIP. QPCR data represent mean SEM (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

CONCLUSION

This study provides a novel insight into the mode of action of PEM showing that this drug counteracts protein biosynthesis when cells have a non-limited access to nutrients in a mechanism that is independent of the endoplasmic reticulum stress signaling. However, in the context of repressed translation by glucose scarcity, PEM treatment dampened UPR activation and partially restored protein synthesis and is associated with a weaker induction of apoptosis.
Beyond the fundamental aspects related to the crosstalk between the therapeutic and metabolic stress integration, our data support the utilization of physiological media to investigate drug efficacy in preclinical studies.

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