eEF2K- and Unfolded Protein Response-mediated Protein Synthesis Following Nelfinavir Treatment in CLL

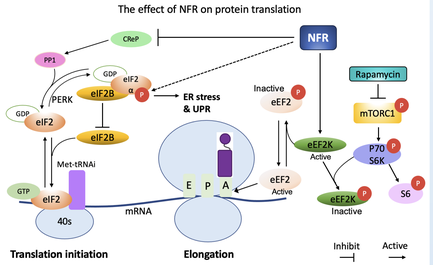
Jiatian Li, Jodie Hay, Karen Dunn, Martin Bushell, Alison M Michie
Paul O'Gorman Leukaemia Research Centre, School of Cancer Science, University of Glasgow, Glasgow, G12 8QQ

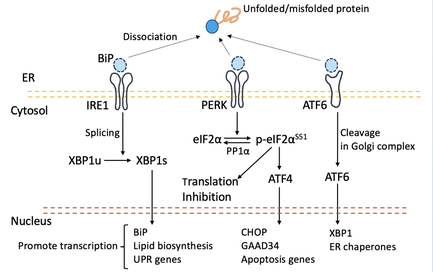
Friends of the Paul O'Gorman Leukaemia Research Centre Glasgow
FUNDED BY Beatson cancer charity

BACKGROUND

Appropriate regulation of protein activity is critical for cell fate decisions: eEF2K, a key regulator of protein translation elongation, inhibits protein synthesis by phosphorylating eEF2^{T56} (p-eEF2^{T56}) to negatively regulate eEF2 and translation elongation, while prolonged unfolded protein response (UPR) can be induced in response to accumulation and disassembly of misfolded/unfolded proteins and regulates cell survival. Nelfinavir (NFR), a safe, oral drug that was originally developed as an HIV-1 aspartyl protease inhibitor, can exert anti-cancer effects by inducing cell cycle arrest, apoptosis and inhibiting protein synthesis, leading to clinical trials for myeloma, prostate, and cervical cancer.

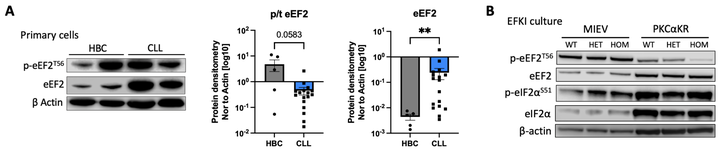
NFR also activates eIF2α-mediated UPR and inhibits eEF2-mediated protein translation processes. Our previous studies demonstrated that treatment of chronic lymphocytic leukaemia (CLL) cells with the mTORC1 inhibitor rapamycin, resulted in eEF2 inhibition (increased p-eEF2^{T56}) via elevated eEF2K activity, leading to reduced MCL1 expression. We aim to investigate whether the eEF2K/eEF2 pathway and UPR can be exploited as a therapeutic target in CLL, through the combination of NFR and first-line treatments, thus providing novel treatment options.





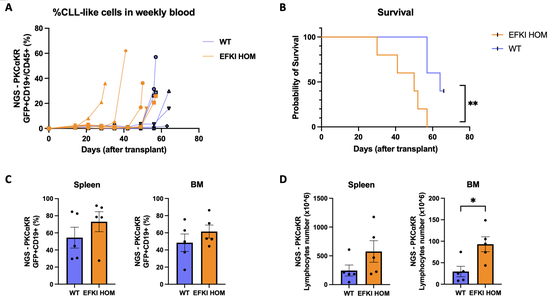
RESULTS

1 eEF2 is more active in primary CLL cells and CLL model



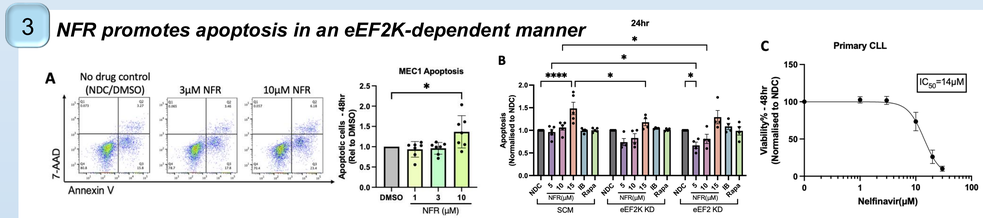
A. Proteins were extracted from heathy B cells (HBC) and primary CLL cells. Western blots show a significant upregulation of total eEF2 in CLL. **B.** BM HSC cells from WT and eEF2K reduced (EFKI) mice were isolated, transfected by MIEV (non-CLL) and PKCαKR (CLL model) and co-culture with OP9 cells to grow into B cells. CLL mice model possessed higher total eEF2 and lower p-eEF2, which confirmed the result in primary CLL. In addition, the CLL model also exhibited lower levels of p/t eIF2α.

2 EFKI HOM CLL drives more aggressive disease in NSG mice



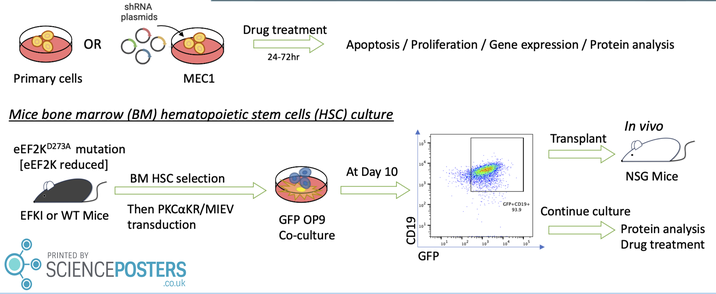
A. WT or EFKI HOM CLL-like cells were transplanted into NSG mice via intravenous injection. The percentage of CLL-like cells among total lymphocytes in peripheral blood was monitored weekly. **B.** Kaplan–Meier survival curves of NSG mice engrafted with WT and EFKI HOM CLL-like cells. Mice transplanted with EFKI HOM CLL-like cells exhibited accelerated disease progression and reduced survival compared with the WT group. **C&D.** At the experimental endpoint, mice engrafted with EFKI HOM cells displayed a higher percentages of CLL-like cells (C) and an increased total lymphocytes (D) in the spleen and bone marrow.

3 NFR promotes apoptosis in an eEF2K-dependent manner

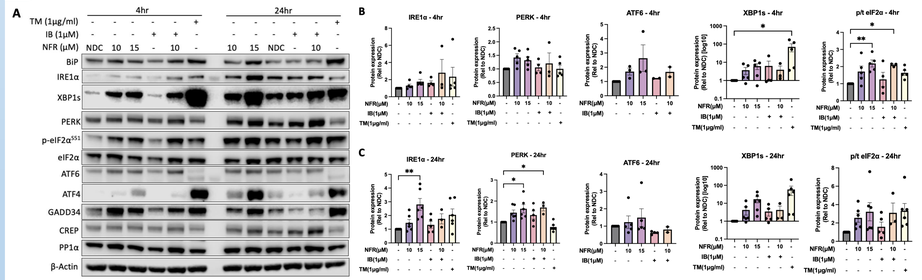


A. MEC1 cells were treated by NFR. After 48 hr, cells apoptosis was detected by Annexin V and 7AAD. 10 μM NFR significantly induced apoptosis (Annexin V+). **B.** Scrambled (SCM), eEF2K knock down (KD) and eEF2 KD MEC1 cells were established by shRNA and confirmed KD by qRT-PCR. Cells were treated by NFR, 1 μM ibrutinib (IB) and 10nM Rapamycin (Rapa) for 24hr. The apoptosis induced by 15 μM NFR was significantly different between SCM and eEF2K KD cells. A significant difference was observed between SCM and eEF2 KD cells following treatment with 5 and 10 μM NFR. 15 μM NFR significantly promote apoptosis only in SCM cells, which demonstrated eEF2K KD and eEF2 KD cells are less sensitive to NFR.
C. NFR induced apoptosis in primary CLL, which IC_{50} is within achievable range in clinical treatment.

MATERIALS & METHODS



4 NFR active UPR through IRE1 and PERK



A. Proteins were extracted from MEC1 cells after 4hr and 24 hr treatment. Tunicamycin (TM) inhibits N-linked glycosylation of protein to induce ER stress and UPR. BiP, IRE1α/XBP1, PERK/eIF2α/ATF4 were upregulated under NFR, which indicated NFR might active UPR mainly through IRE1 and PERK. The response was initiated at 4 hours and sustained for 24 hours.

CONCLUSIONS

- eEF2 is more active in CLL and in the mouse CLL model, supporting the observed increases in protein synthesis in our CLL models.
- EFKI HOM CLL cells drive accelerated disease progression and increased lymphocyte burden in NSG mice.
- NFR induces apoptosis and inhibits proliferation in the MEC1 cells at clinically achievable concentrations.
- NFR increases eEF2 phosphorylation, and first-line therapy enhances its ability to significantly induce apoptosis and suppress proliferation in MEC1 cells.
- NFR-induced apoptosis is dependent on the eEF2K/eEF2 signalling axis, as indicated by the reduced apoptosis in both sh-eEF2K and sh-eEF2 knockdown MEC1 cells compared with SCM cells, upon treatment with NFR.
- NFR triggers UPR mainly through IRE1α/XBP1 and PERK/eIF2α/ATF4 pathways.