Nelfinavir Inhibits the TCF11/Nrf1-Mediated Proteasome Recovery Pathway in Multiple Myeloma

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*This research is published by Fassmannová et al. 2020, Cancers **2020**, 12(5), 1065; https://doi.org/10.3390/cancers12051065*

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Introduction

Proteasome inhibitors are the backbone of multiple myeloma therapy. However, disease progression or early relapse occur due to development of resistance to the therapy. One important cause of resistance to proteasome inhibition is the so-called bounce-back response, a recovery pathway driven by the TCF11/Nrf1 transcription factor, which activates proteasome gene re-synthesis upon impairment of the proteasome function. Thus, inhibiting this recovery pathway potentiates the cytotoxic effect of proteasome inhibitors and could benefit treatment outcomes. DDI2 protease, the 3D structure of which resembles the HIV protease, serves as the key player in TCF11/Nrf1 activation. Previous work found that some HIV protease inhibitors block DDI2 in cell-based experiments. Nelfinavir, an oral anti-HIV drug, inhibits the proteasome and/or pAKT pathway and has shown promise for treatment of relapsed/refractory multiple myeloma. Here, we describe how nelfinavir inhibits the TCF11/Nrf1-driven recovery pathway by a dual mode of action. Nelfinavir decreases the total protein level of TCF11/Nrf1 and inhibits TCF11/Nrf1 proteolytic processing, likely by interfering with the DDI2 protease, and therefore reduces the TCF11/Nrf1 protein level in the nucleus. We propose an overall mechanism that explains nelfinavir's effectiveness in the treatment of multiple myeloma.

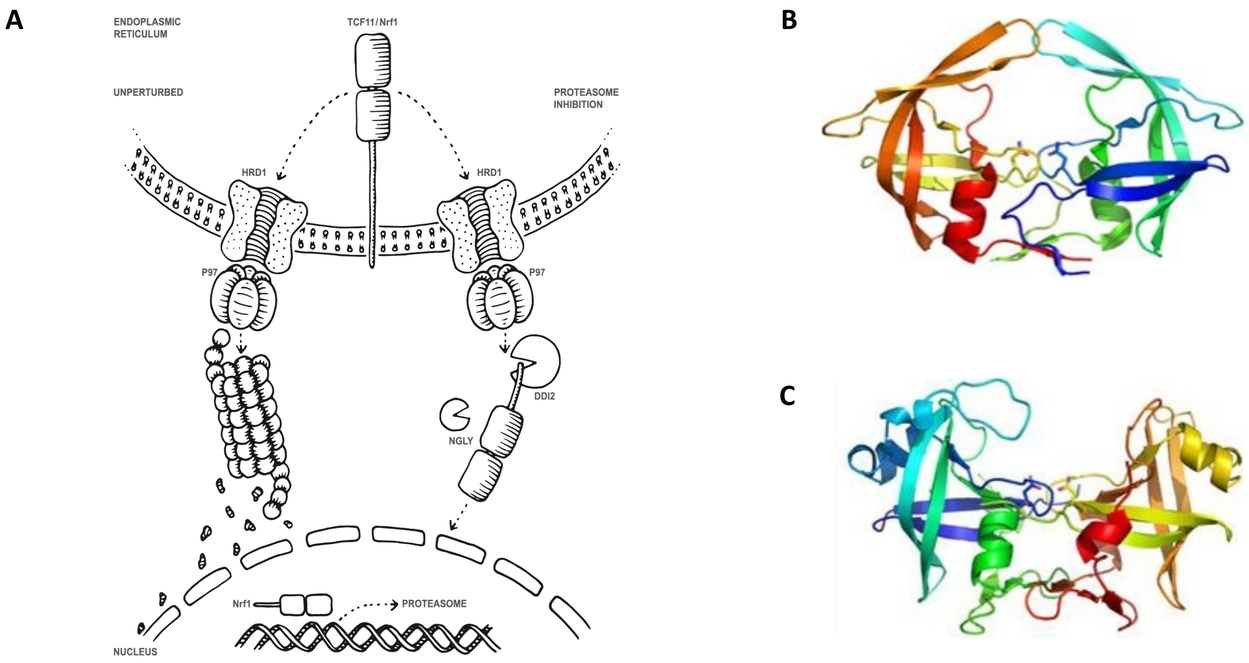


Fig. A: TCF11/Nrf1 is a transcription factor responsible for regulating genes involved in protecting cells from oxidative stress and regulating proteasomal activity. **B:** HIV-1 protease. **C:** DDI2 RVP domain

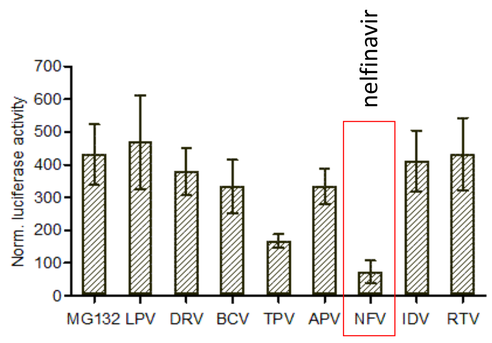
Methods

- The luciferase reporter cell line (3xPSMA4-ARE-Luc) sensing the level of activated TCF11/Nrf1 after proteasomal inhibiton
- GFP degron reporter cell line (UbG76V-GFP) were used to evaluate the effect of HIV protease inhibitors on TCF11/Nrf1 pathway and the overall proteasome re-synthesis capacity
- Multiple myeloma cell lines OPM-2 and RPMI8226, HEK293 and HEK293T were subsequently used for deeper analysis of the TCF11/Nrf1 pathway – treatment with bortezomib and nelfinavir
- qPCR for levels of proteasomal subunits, Nrf1 and Nrf2
- Protein levels of Nrf1 and its processing were estimated using immunoblot assay
- The high throughput confocal microscopy for translocation of Nrf1 protein into the nucleus

Results

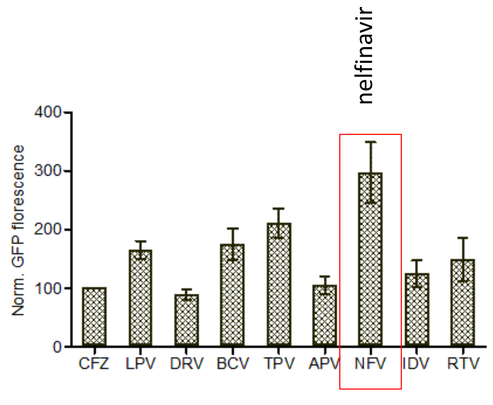
1. Efficient proteasome re-synthesis can be attenuated by HIV PI nelfinavir.

Luciferase assay reporting TCF11/Nrf1 transcriptional activity. The reporter cells were co-treated with 1μM MG132 and 10μM HIV PIs. At 16 h post-transfection, a dual luciferase assay was used to measure luciferase activity (the lower signal, the higher inhibition).

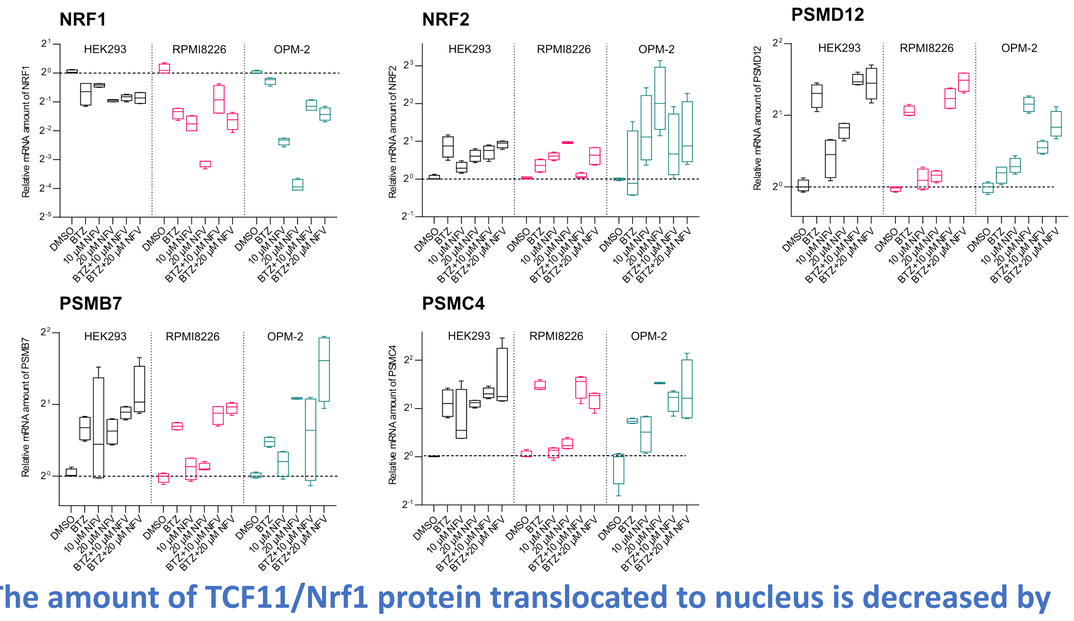


2. Efficient proteasome re-synthesis can be attenuated by HIV PI nelfinavir.

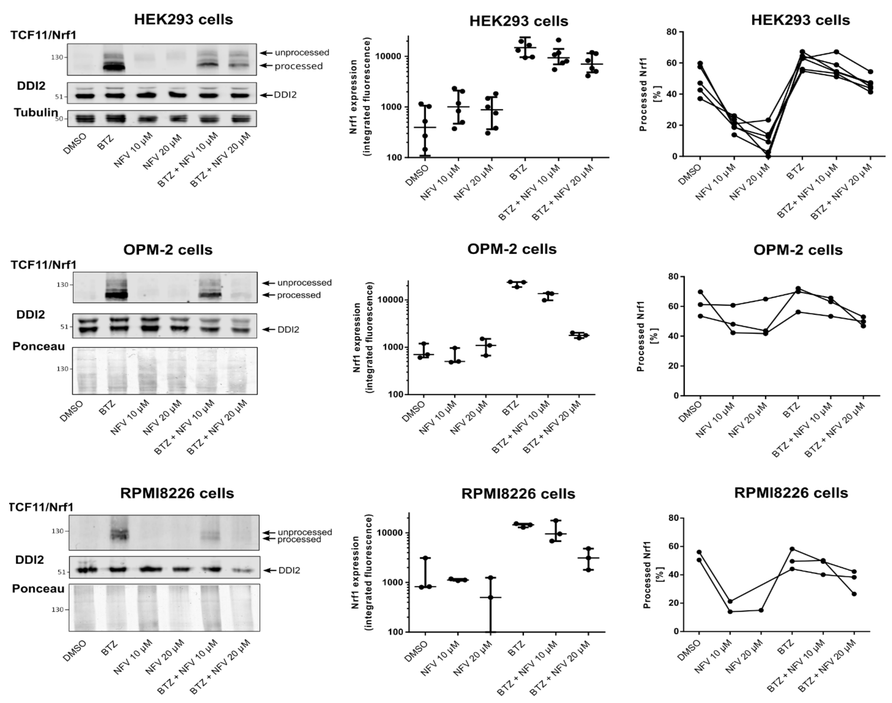
Screening of HIV PIs with an N-end rule GFP reporter assay to measure proteasome activity. U2OS cells stably expressingUbG76V-GFP reporter were treated with 200 nM CFZ for 2 h. The cells were washed with the PBS and treated with HIV PIs at 10μM. The GFP fluorescence (dependent on proteasome activity) was measured 24 h after HIV PI treatment and normalized to the CFZ-treated cell.



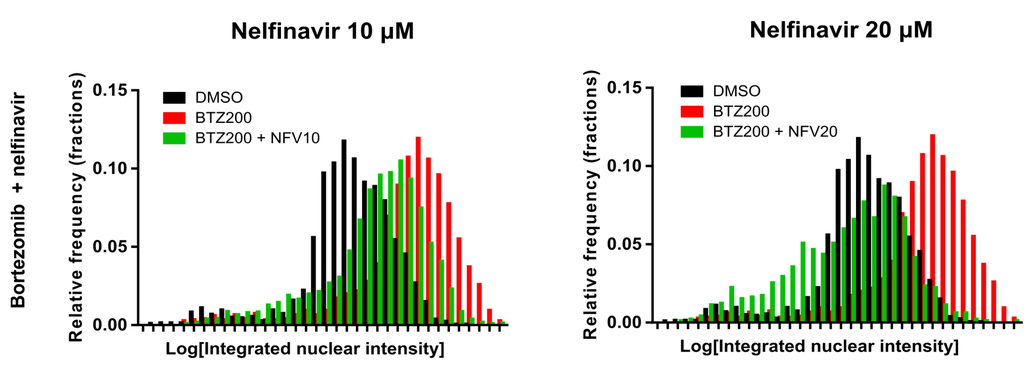
3. Nelfinavir decreases TCF11/Nrf1 gene expression and activates Nrf2.



4. Nelfinavir Inhibits Proteolytic Processing of TCF11/Nrf1 in MM Cells



5. The amount of TCF11/Nrf1 protein translocated to nucleus is decreased by combinational of nelfinavir + bortezomib.



Conclusions

Nelfinavir
- inhibits TCF11/Nrf1 mediated protesome re-synthesis
- decreases the active form of SREBP transcription factor by inhibition S2P protease and TCF11/Nrf1 transcription factor
- decreases the level of TCF11/Nrf1 in nucleus and proteasome re-synthesis
- at high concetration inhibits β1/β5 and β2 activity of the proteasome

Support
- MEYS of the Czech Republic within National Sustainability Program II [Project BIOCEV-FAR LQ1604]
- „BIOCEV" project (CZ.1.05/1.1.00/02.0109)
- Czech League Against Cancer and the Charles University Grant Agency (grant #1172119)