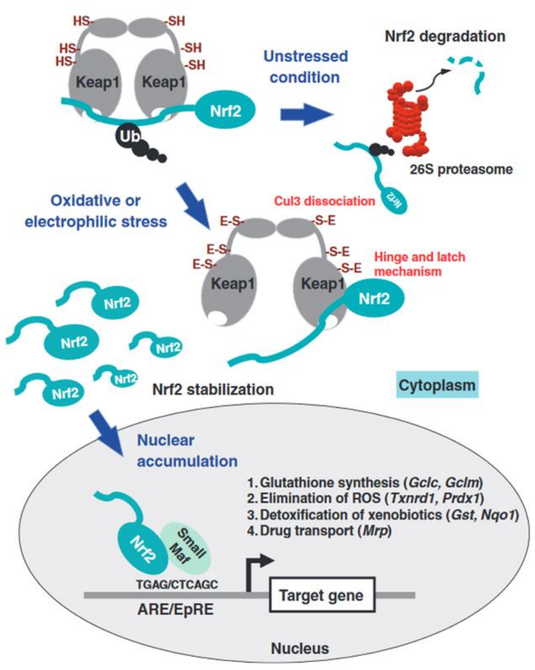
Nrf2 in non-small cell lung cancer: potential role in immune surveillance

Alicja Sznarkowska [1#], Sara Mikac [1], Robin Fahraeus [1,2,3,4]

[1] *International Centre for Cancer Vaccine Science University of Gdansk Wita Stwosza 63, 80-308 Gdansk.* [2] Université Paris 7, Inserm, UMR 1162, Paris, France.
[3] Department of Medical Biosciences, Umeå University, Umeå, Sweden. [4] RECAMO, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech Republic.

[#] ***alicja.sznarkowska@biotech.ug.edu.pl***

Background and aim



Transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) is a key regulator of cytoprotective response to oxidative and xenobiotic stresses.

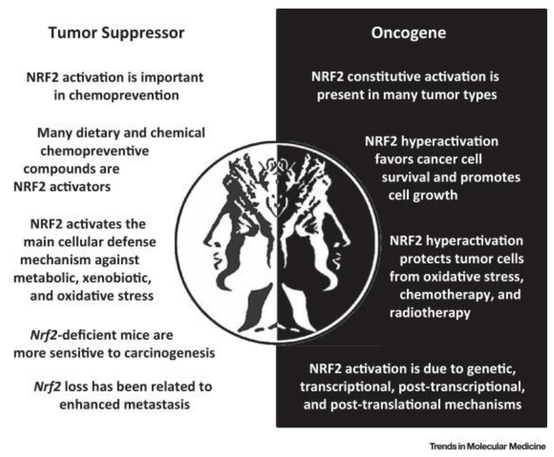
Activated Nrf2 induces expression of **plethora of cytoprotective genes**: detoxification enzymes, antioxidants, antiinflammatory intermediates, drug export pumps.

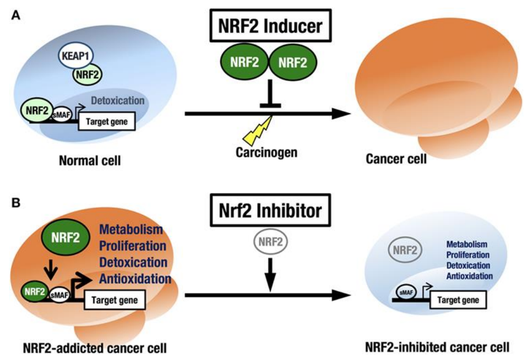
At steady state, Nrf2 is kept inactive in the cytosol by its **inhibitor protein Keap1** (Kelch-like ECH-associated protein 1), which targets Nrf2 for proteasomal degradation.

Nrf2 constitutive activation is present in many tumor types, including non-small cell lung cancer.

Two different strategies for cancer therapy focused on Nrf2: induction of Nrf2 in normal cells for chemoprevention and inhibition of Nrf2 in Nrf2-addicted malignant cancer cells

Nrf2 role in immune response in both normal and cancer cells has just began to be elucidated.





Does Nrf2 influence MHC class I levels in non-small cell lung cancer cells?

Methods

Model: two NSCLC cell lines of different basal Nrf2 levels: Nrf2-addicted adenocarcinoma **A549** (Keap1 mutation) and squamus lung carcinoma **RERF** LC in comparison to normal lung fibroblasts (**NLF**)

Knockdown of Nrf2 and Keap1 was performed with 10nM siRNA. Proliferation was measured with the use of xCELLigence platform (ACEA Biosciences). 24 h after knockdown cells were counted and seeded 10^4/well x in e-plate (7 wells/each condition) and the proliferation was measured in real time for 72 h.

Crystal violet staining was performer 24 h after treatment of cells seeded at 3x10^5 cells/well and treated with 1 µM Cucurbitacin B. Cells were pretreated with 5mM N-acetyl-L-cysteine were indicated or treated with 5 µM ML385 (Nrf2 inhibitor).

MHC class I (HLA-A, -C) levels were analysed 48 h upon Nrf2 knockdown by Western blot.

Conclusions

We show here that transcription factor Nrf2 promotes proliferation of non-Nrf2 addicted non-small cell lung cancer cell line RERF LC.

Nrf2 inhibition potentiates action of ROS-inducing anticancer compound Cucurbitacin B.

Interestingly, in normal lung fibroblasts, **silencing of Nrf2 expression reduced MHC class I levels**, indicating that Nrf2 might be important not only for the growth and protection of cells but also in terms of immune surveillance. This Nrf2 effect is absent in cancer cell lines.

Discussion

Growing body of evidence indicates the role of Nrf2 in cancer cells reprogramming and progression. Its much more than just conferring protection agains oxidative stress. In multiple cancers Nrf2 was shown to promote growth, induce metabolic reprograming and very recently, to modulate anticancer immune response. **This study points to the possible link between Nrf2 and MHC class I. We hypothesise that Nrf2 positively regulates MHC class I levels in normal cells. This regulation is lost in cancer cells which downregulate their MHC class I levels to escape immune surveillance**. In our research we explore the Nrf2-MHC c. I interplay and ask the following questions:

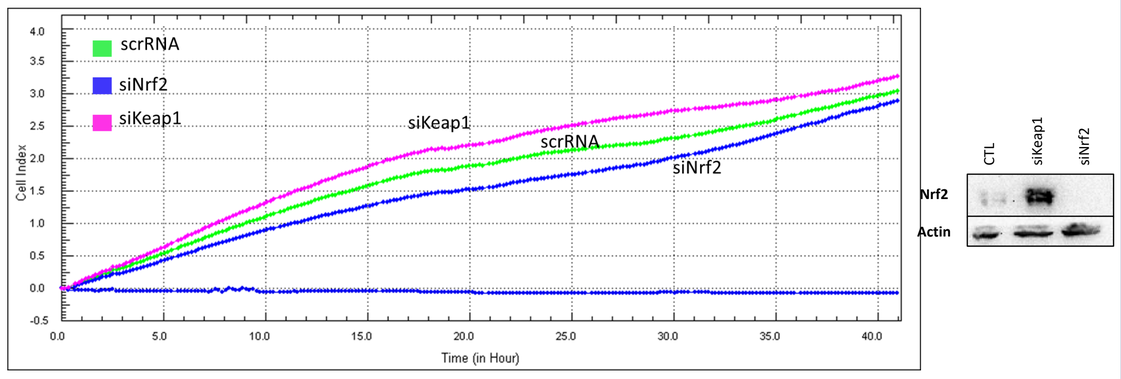
Why is Nrf2 effect on MHC c. I present only in non transformed cell line?

Is it direct (Nrf2 regulates MHC c. I transcription or protein/mRNA stabilization) or indirect effect?

What could be the effect of Nrf2 activators and inhibitors on the immune system?

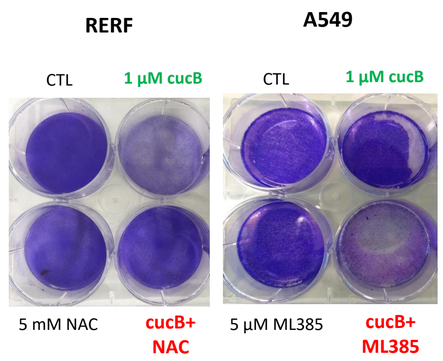
Results

Keap1 KD results in Nrf2 accumulation and boosts proliferation of RERF cell line. On the contrary, silencing of Nrf2 slows down RERF proliferation.

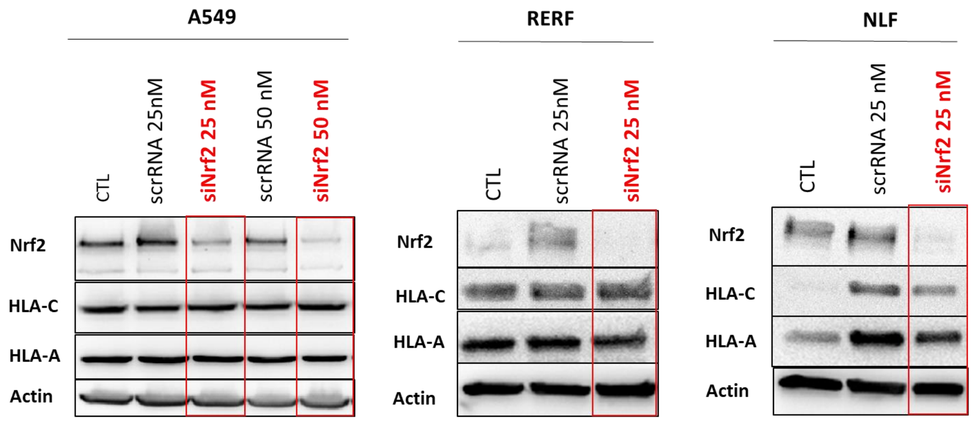


Cell proliferation measured by xCelligence platform.
Each line is an average of 7 repeats.

Cucurbitacin B (CucB) is a ROS-inducing natural compound effectively killing cancer cells. NAC pretreatment alleviates CucB effect. Nrf2 inhibitor ML385 potentiates CucB action.



Nrf2 deficiency decreases MHC class I levels in normal lung fibroblasts



This work was supported by National Science Centre PRELUDIUM 9 grant no 2015/17/N/NZ3/03773.