Nrf2 role in immune surveillance

Sara Mikac, International Centre for Cancer Vaccine Science,

Intercollegiate Faculty of Biotechnology of University of Gdańsk and Medical University of Gdańsk

Supervisors: Dr Alicja Sznarkowska, Prof Robin Fahraeus

The Nrf2–Keap1 signaling pathway (nuclear factor erythroid 2 [NF-E2]-related factor 2; Kelch-like erythroid cell-derived protein with CNC homology [ECH]-associated protein 1) is one of the most important cell defense and survival pathways. Nrf2 belongs to the Cap 'N' Collar (CNC) family that contains a conserved basic leucine zipper (bZIP) structure. The main function of Nrf2 is to activate the cellular antioxidant response by inducing the transcription of a wide array of genes that are able to combat the harmful effects of extrinsic and intrinsic insults, such as xenobiotics and oxidative stress. Detailed mechanistic studies indicate that Keap1 is the molecular switch that controls activation and inactivation of the Nrf2 pathway.

Nrf2 was regarded for a long time as a tumor inhibitor, thus providing the rationale of tumor-prevention strategies using Nrf2 activators. However, this view has to be modified because it was recognized that deregulated Nrf2 activity can also favor a malignant phenotype and promote cancer. Accumulation of Nrf2 in cancer cells creates an environment conducive for cell growth and protects against oxidative stress, chemotherapeutic agents and radiotherapy. The prognosis of patients with tumors expressing high levels of Nrf2 in the clinic is poor partly due to Nrf2's ability to enhance cancer cell proliferation and promote chemoresistance and radioresistance. It appears that transient activation of Nrf2 in normal cells (where the Nrf2–Keap1 axis is intact) is protective; however, constitutive activation of Nrf2, as seen in cancer, enhances the survival and progression of cancer cells.

Based on the previous observations that Nrf2-Keap1 signaling pathway has a potential role in immune surveillance, the main aim of my research is to investigate the role of transcription factor Nrf2 in MHC class I expression in normal vs. lung cancer cells. For that purpose, I am using human normal lung fibroblasts derived from anencephalous embryo and non-small cell lung cancer cell line A549, a lung adenocarcinoma, with a somatic mutation of KEAP1 gene at G333C. It is a typical cancer cell line that exhibits aberrantly active Nrf2. To verify if MHC class I expression is indeed related with Nrf2 in A549 cell line, I made use of CRISPR/Cas9-induced NRF2 knockout in A549 cells (clone 2-11). The CRISPR-directed gene-editing system was designed to disable the transactivation Neh5 domain of Nrf2 bearing nuclear export signal (NES), which reduces the capacity of the protein to re-enter the nucleus.

To explore the effect of Nrf2 on MHC class I expression in normal lung fibroblasts and lung cancer cells, I performed analysis on transcriptional, protein and cell surface level and the results showed that depletion of Nrf2 decreased MHC class I protein and cell surface level, in normal lung fibroblasts and in non-small cell lung cancer cell line (A549) with functional knockout of Nrf2. Interestingly, this effect was not observed on transcriptional level where the depletion of Nrf2 increased the expression of MHC class I, in both normal lung fibroblasts and A549 cells. It leads to the assumption that Nrf2 can regulate translation of MHC class I molecules, or affect degradation of HLAs. I will perform further analysis to answer this question.

Results obtained so far indicate that Nrf2 functions not only as a transcription factor and this transcription-independent role is important in immune surveillance.