Abstract

The transcription factor Nrf2 is recognized for its pro-survival and cell-protective role upon exposure to different types of extrinsic and intrinsic insults. It controls a number of cellular processes such as proliferation, differentiation, apoptosis, autophagy, protein homeostasis and amino acids metabolism. Under no-stress conditions, the level of Nrf2 is low since it is constantly degraded by Keap1-Cul3-E3 ubiquitin ligase pathway. However, when the regulation of Nrf2 is imbalanced (e.g. via oncogene activation or mutations), it becomes constitutively active promoting carcinogenesis, metastasis and radio- and chemoresistance. Transient activation of Nrf2 in normal cells is protective, however, constitutive activation as seen in cancer, enhances the survival and progression of cancer cells. Since oncogenic and immune pathways are interconnected, my PhD project investigated the impact of Nrf2 on the expression of major histocompatibility complex class I (MHC-I) molecules, which present self and non-self peptides to the immune cells. In the course of the project we have also identified the stable, non-canonically regulated Nrf2 which does not play a transcription factor role.
In the first part of the project, we have shown that Nrf2 knockdown in both, normal lung fibroblasts and the non-small cell lung cancer cell line A549, reduced intracellular and cell surface MHC-I molecules levels, but not their transcript levels. Inhibition of translation with emetine revealed that Nrf2 stabilizes MHC-I in cells, while labeling of freshly synthesized proteins with Click-iT chemistry indicated that Nrf2 could also affect their synthesis. Immunoprecipitation studies together with molecular modeling and molecular dynamics simulations showed that Nrf2 binds to MHC-I and stabilizes it in cells.

The second part of the thesis focuses on the identification and characterization of the stable, non-canonically regulated Nrf2 isoform (named ΔN-Nrf2) that is abundantly expressed in the lung cells. This form originates from the alternatively transcribed *NFE2L2* transcripts and is not degraded via Keap1-Cul3-mediated pathway. Compared to the full-length Nrf2, ΔN-Nrf2 has a deletion of the first 16 amino acids causing the impairment of the Keap1 binding. ΔN-Nrf2 is localized in the cytoplasm under homeostatic conditions and upon exposure to electrophilic stress, therefore it does not play a transcription factor role.

Altogether these results point to the new function of Nrf2 in cells, which relies on the protein-protein interaction.