'The dark side of Nrf2' A metabolomic pilot study about the role of the transcription factor NRF2 in glycolysis, in KrasG12D-driven non-small cell lung cancer mouse model

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Increasing attention has been paid to the role of Nrf2 in cancer cells because the constitutive stabilization of NRF2 has been observed in many human cancers that have poor prognoses. It was reported in 2006 that in some lung cancer patient samples and lung cancer cell lines, Nrf2 is constitutively localized into the nucleus as a result of missense mutations in KEAP1. In Nrf2 overexpressing cancers, the elevated expression of cytoprotective genes confers growth and survival advantages. Cancers with elevated Nrf2 activity are more resistant to chemo- and radiotherapy. Moreover, these cancer cells are able to evade apoptosis and exhibit higher rates of proliferation, though it is unclear why the latter occurs. It has been shown that some oncogenes are able to increase the basal Nrf2 expression, activate Nrf2-mediated antioxidant response, and lower the cellular ROS levels in primary murine cells. Given that cancer cells are subject to metabolic reprogramming to support rapid synthesis of macromolecules, it is also possible that Nrf2 promotes tumor cell proliferation by influencing intermediary metabolism. Nrf2 promotes the generation of building blocks for macromolecules synthesis and cancer cell proliferation by either directly or indirectly regulating genes involved in glutathione (GSH) biosynthesis and recycling, NADPH production, and Pentose Phosphate Pathway (PPP) activation, suggesting the transcription factor represents a dominant controller of metabolic flux through the PPP. However, the mechanisms with which Nrf2 accelerates proliferation are not fully understood. In order to understand the role of NRF2 in lung cancer metabolism, we analyzed how glucose metabolism changes in response to KEAP1 deficiency-induced NRF2 overactivity, in KrasG12D driven mouse model of non-small cell lung cancer (NSCLC), using Stable Isotope Resolved Metabolomics (SIRM) approach. Does NRF2 accelerate cancer cell proliferation by reprogramming glucose metabolism in order to fuel Pentose Phosphate Pathway? To address the aforementioned question, we used LSL-Kras G12D and LSL-Kras G12D+LKB1-/- -driven NSCLC mouse models, with NRF2 overactivity induced by KEAP1-/- (Genetically Engineered Mouse Models, AdenoCre activation). The analysis has been done by applying the SIRM approach, a combined use of stable isotope tracers and metabolomic analysis (NMR, GC-MS), comparing the metabolic profiles of the six different mice groups. The NMR spectra analysis, for the mice groups with NRF2 overactivity (in particular KEAP1-/-) , suggests a potential role for NRF2, as responsible for enhancing glycolysis, expression of genes involved in GSH biosynthesis/recycling, NADPH production, and PPP activation. Analyzing the GC-MS data, the flux analysis (fractional enrichment) shows us conflicting results for the same groups. Based on these observations and because of the lack of statistically significant data, we can just formulate some hypothesis: most of the glucose that enters into the cells is metabolized via PPP, and used for nucleotides, nucleic acids, aromatic amino acids synthesis and NADPH generation; the glycolytic pathway relies on another source of fuel, as glycogen, or maybe, LKB1-/- affects glucose metabolism.