Epigenetic regulation of NRF2-NQO1 axis in human head and neck-originating cancer cell lines and untransformed fibroblasts exposed to nutritional stress

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Introduction

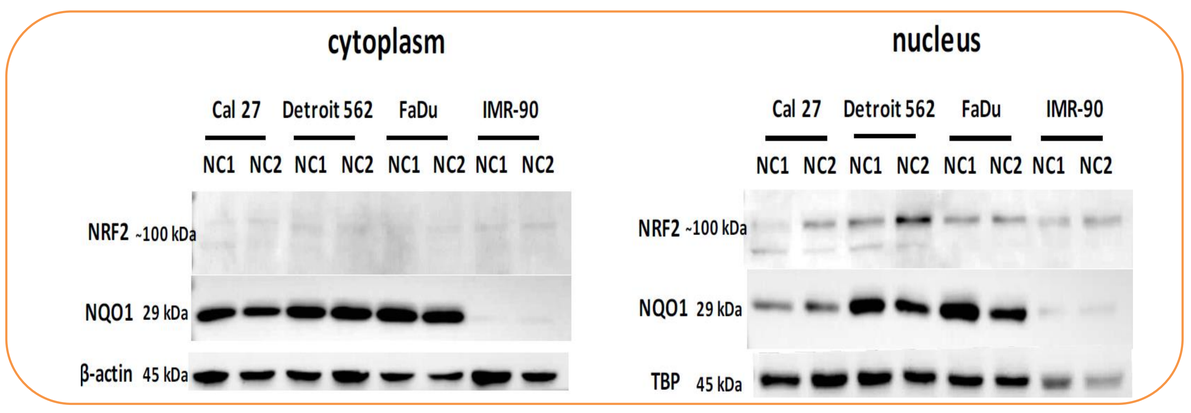
Nutritional stress leads to an disbalanced cellular redox status that is characterized by an increased level of Reactive Oxygen Species (ROS). This disbalance is manifested through multiple molecular changes which significantly affect numerous cellular functions, including proliferative and apoptotic pathways. Cellular antioxidative machinery relies heavily on potent detoxifying mechanisms. One of the central molecules involved in ROS removal is Nuclear Factor Erythroid 2 Like 2 (NRF2) transcription factor [1]. When there is no excess of ROS, the majority of NRF2 is in the cytoplasm, bound to its protein partner Kelch-like ECH-associated protein 1 (KEAP1). Important consequences of ROS excess is the release of NRF2 from KEAP1, its entrance into the nucleus and its binding to the antioxidant response elements (ARE) present in the promoter regions of NRF2 target genes. One of NRF2 target genes is NAD(P)H: Quinone Dehydrogenase 1 (*NQO*1). Successful triggering of transcription depends on many factors, including spatial chromatin accessibility. In this research, we explored the NRF2-NQO1 axis at the transcriptional and protein level, under three different nutritional conditions. The data obtained were associated with the presence of a highly suppressive chromatin mark – trimethylated histone H3 at lysine 27 (H3K27me3) [2], at the NRF2 binding site in the *NQO*1 promoter.

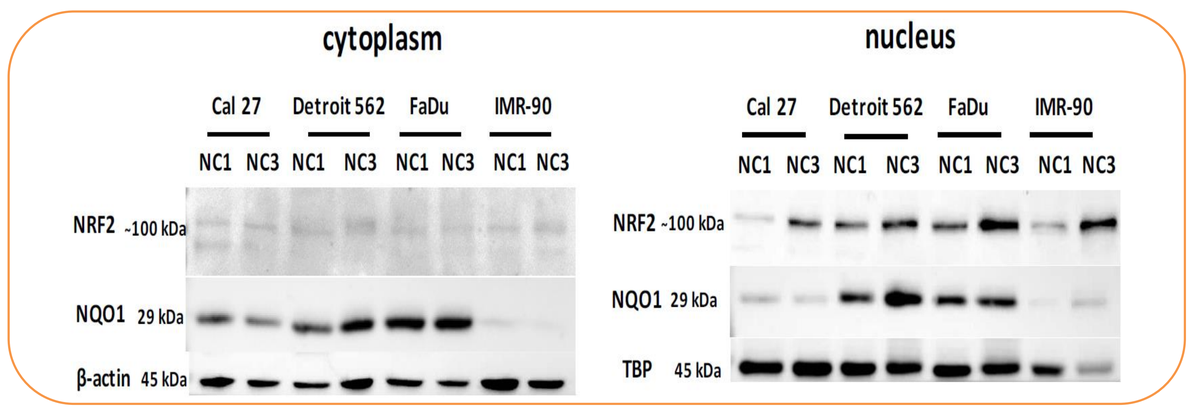
Materials and Methods

Three head and neck-originating cancer cell lines - Cal 27, Detroit 562, FaDu, and immortalized fetal lung fibroblasts, IMR-90, were cultured for 48 hours in DMEM, under the three nutritional conditions with respect to glucose and glutamine (Table 1). Regarding NRF2 and NQO1, nuclear and cytoplasmic protein fractions were analyzed by Western blot, while the transcripts were quantified by TaqMan Real-time PCR. Chromatin immunoprecipitation (ChIP) was used for estimating the amount of NRF2 and H3K27me3 bound to *NQO*1 promoter. The data obtained was analyzed with 1-way ANOVA and Tukey post-hoc test. The statistical significance of the differences obtained for all data analyzed was considered significant at $p < 0.05$.

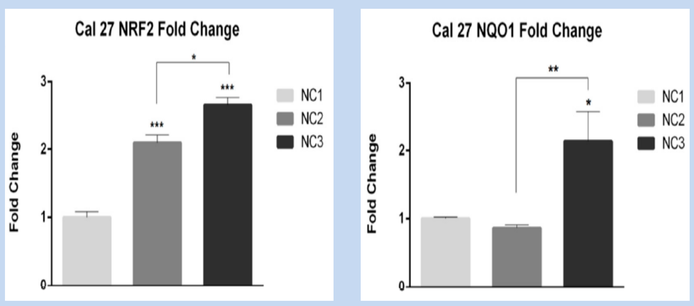
Table 1. Nutritional conditions with respect to glucose and glutamine

Nutritional conditions	Description
NC1	high glucose (4,5 g/L) + glutamine (0,584 g/L)
NC2	low glucose (1 g/L) + glutamine (0,584 g/L)
NC3	traces of glucose and glutamine provided by fetal bovine serum

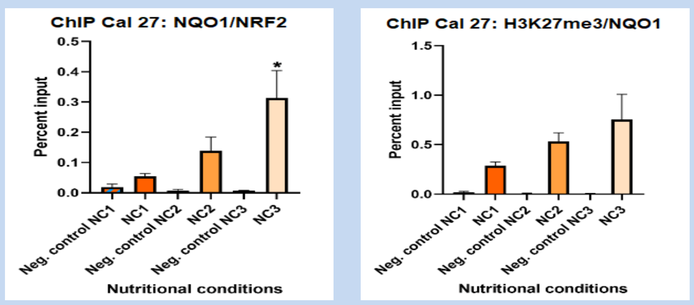




Cal 27



RT-qPCR: NRF2 and NQO1



ChIP Cal 27: NRF2 and H3K27me3 bound *to NQO*1 promoter

Results and Discussion

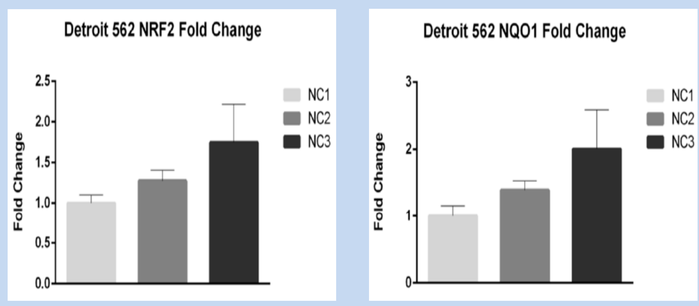
According to WB, under NC3, a significant increase of NRF2 was recorded in the nucleus of the three cell lines (NC1 vs NC3: Cal 27 p=0.039, Detroit 562 p=0.0306, IMR-90 p=0.0103). However, under this extreme condition, it was not present in FaDu. In this cell line, ChIP revealed a significant amount of NRF2 bound to *NQO*1 promoter under NC1, and decrease of NRF2 binding associated with H3K27me3 enrichment, under NC3. Increased nuclear entrance of NRF2 in Detroit 562 and IMR-90 under NC3 did not result with increase of NRF2 bound to *NQO*1 promoter.

The increase of *NQO*1 transcript in Cal 27 under NC3 was the strongest among all cell lines tested [3], in line with enrichment of NRF2 bound to *NQO*1 promoter. Unexpectedly, under NC3, an increase of H3K27me3 was also recorded in Cal 27. In Detroit 562, nutritional stress led to modest increase of *NQO*1 transcription (NC1<NC2<NC3). However, the expected promoter enrichment with NRF2 was absent, especially under NC3. Under this condition, the level of the *NQO*1 transcript was higest, associated with the highest enrichment with H3K27me3 and the lowest amount of bound NRF2. Thus, in respect to H3K27me3 enrichment and the NRF2 binding to *NQO*1 promoter, FaDu and Detroit 562 show a high level of similarity. The situation in Cal 27 is the opposite, but only in respect to the NRF2 binding.

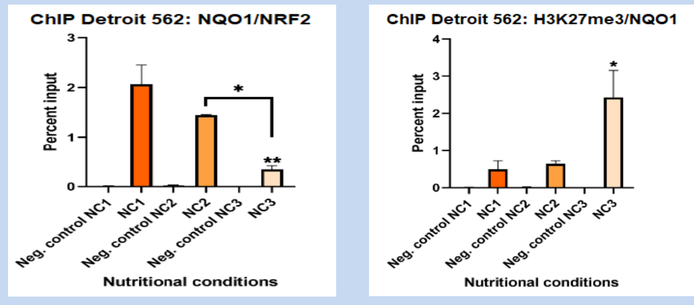
Surprisingly, a similar scenario similar to those obtained in FaDu was observed in the untransformed fetal fibroblasts where, under NC3, *NQO*1 transcription increased, although ChIP recorded a statistically decreased level of NRF2 binding. The level of H3K27me3 in IMR-90 seemed to be unchanged. However, due to the strong signal obtained from negative control samples, it is, for now, just at the level of „suggestive".

Our results show that, although the NRF2-NQO1 axis is considered to be highly conserved, the mechanisms included in its activation seem to significantly vary and are cell-type specific.

Detroit 562

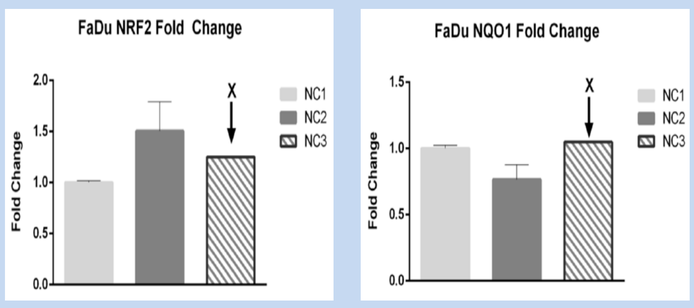


RT-qPCR: NRF2 and NQO1



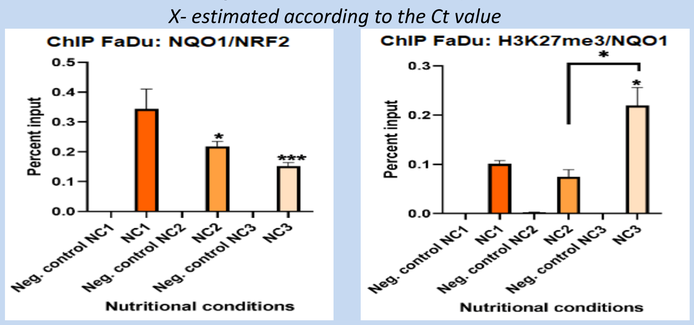
ChIP Detroit 562: NRF2 and H3K27me3 bound to *NQO*1 promoter

FaDu



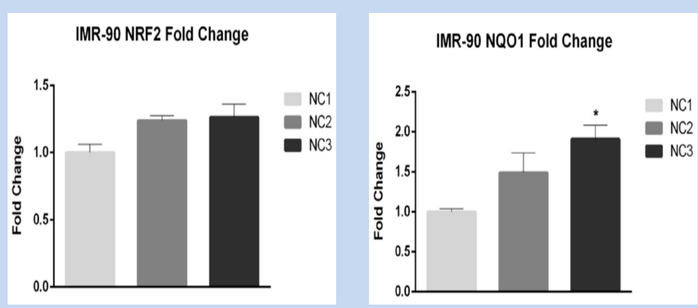
RT-qPCR: NRF2 and NQO1

X- estimated according to the Ct value

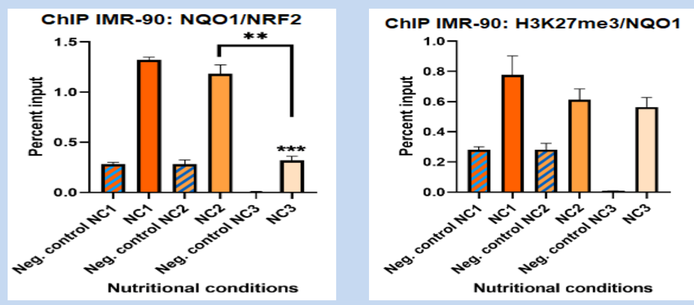


ChIP FaDu: NRF2 and H3K27me3 bound *to NQO*1 promoter

IMR-90



RT-qPCR: NRF2 and NQO1



ChIP IMR-90: NRF2 and H3K27me3 bound to *NQO*1 promoter

Acknowledgement

This research has been entirely supported by the Croatian Science Foundation, Project No. 4404, CrossEMPATICNRF2 (PI: Koraljka Gall Trošelj)

References

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