Abstract

Iron is an essential trace element required for many processes within a cell, including DNA synthesis and cell cycle progression. Moreover, it is critical for cellular respiration in mitochondria. Due to their proliferative nature, cancer cells are dependent on iron, and depleting this element *via* iron chelators results in the inhibition of ribonucleotide reductase, leading to cell cycle arrest and apoptosis of cancer cells. Recently, an alternative mechanism for the effect of iron chelators have been proposed, including induction of N-myc downstream regulated gene 1 (*NDRG1*) expression and its inhibitory effect on c-MET, EGFR, and NF-κB pathways, which can act as oncogenes in a certain context.

NDRG1 is a tumour suppressor gene, which is downregulated in many cancers and its downregulation correlates with cancer progression, poor differentiation, and higher metastatic potential. It has been shown that NDRG1 expression can be regulated by intracellular iron – a decrease in intracellular iron leads to upregulation of NDRG1 at mRNA and protein level *via* the HIF-1-dependent mechanism by inhibiting prolyl hydroxylases.

Recently, we have conceived the concept of mitochondrially targeted chelators as an effective anti-cancer agent and in this work, we focused on the evaluation of mitochondrially targeted deferoxamine (mitoDFO) and deferasirox (mitoDFX) on NDRG1 induction in MCF7 breast cancer cells and compared this effect with non-malignant MRC5 fibroblasts. Our results show that induction of NDRG1 does not correlate with the cytostatic and cytotoxic potency of the chelators. In addition, we evaluated the role of tested iron chelators on selected signalling pathways (EGFR, NF-kB, and c-MET) that are affected by NDRG1, and we found that the level of nuclear p-NFκB Ser^{536} was reduced in MCF7 cell line, while EGFR and c-MET were downregulated only in MRC5 cells. To better understand the role of NDRG1 in tumour cells, we constructed MCF7 *NDRG1* knockout clones and showed that the deletion of the *NDRG1* gene changed the phenotype of MCF7 cells. However, it did not affect the response to the tested chelators. Finally, we have shown that mitochondrially targeted iron chelators might induce immunogenic cell death as evidenced by an increase in calreticulin-positive cells after exposure to these chelators. Overall, it seems that NDRG1 induction is not the critical mechanism that would dictate the efficacy of iron chelators, but it rather reflects their ability to induce “pseudohypoxia” by iron deprivation.

Keywords: NDRG1, MCF7, MRC5, iron, iron chelators, signalling pathways, knockout