Adaptive Changes in Tumor Cells in Response to Reductive Stress

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Reductive stress is characterized by an excess of cellular electron acceptors and can be linked with a number of human pathologies including increased cytotoxicity in cancer cells. In order to characterize cellular adaptations to reductive stress, we used stepwise, incremental selection to generate melanoma cell lines with acquired resistance to rotenone (ROTR, 5-fold), n-acetyl cysteine, (NACR, 2-fold), or dithiothreitol (DTTR, 23-fold) or rotenone (ROTR, 5-fold). Cells divided more rapidly in resistant lines and intracellular homeostatic redox-couple ratios (e.g., glutathione, NADPH) were shifted towards the reduced state. Resistance caused alterations in general cell morphology, but only ROTR cells had significant changes in mitochondrial morphology (higher numbers, and more swollen; isolated and more fragmented; decrease in networks; greater membrane depolarization). These changes were accompanied by lower basal oxygen consumption and maximal respiration rates and a more marked reliance on glycolysis for energy production. Whole cell flux analyses and mitochondrial function assays showed that NACR and DTTR preferentially utilized TCA cycle intermediates, while ROTR used ketone body substrates such as D, L, b-hydroxybutyric acid. While NACR and DTTR each resistant line cells had constitutively elevated decreased levels of reactive oxygen species (ROS), in ROTR these were increased. This was accompanied by and spuriously inhibited/activated Nrf2, with concomitant decreased/increased expression of downstream gene products, such as glutathione S-transferase P in ROTR/NACR and DTTR. Adaptations to reductive stress also included enhanced expression of proteins expressing proteins controlling the unfolded protein response (UPR). These included BiP, PDI, CHOP, ATF4, ATF6 and PERK and although expression patterns of these UPR proteins were distinct between the resistant cells, there was an enhancement of expression implying that resistance to reductive stress accompanies a constitutively increased UPR phenotype in each line, but does not result in cell death. Overall, while most tumor cell lines adapt to survive conditions of high oxidative stress, they are also flexibly capable of adapting various pathways to regulate growth and survival in conditions concentrations of drugs that would cause cell death through reductive stress.

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