Title
Microscopic (FLIM) analysis of NADH state changes during cell cycle progression in cancer cells.

Authors
Goul, C
Murata, M
Digman, MA
et al.

Publication Date
2014

P1516

Microscopic (FLIM) analysis of NADH state changes during cell cycle progression in cancer cells.

C. Goul[1], M. Murata[2], M.A. Digman[1,2], S. Rafelski[1];
[1]Developmental and Cell Biology, University California-Irvine, Irvine, CA, [2]Biomedical Engineering, University California-Irvine, Irvine, CA

Cancers exhibit high metabolic activity via the "Warburg effect", the shift to a high rate of glycolysis followed by lactic acid fermentation in the cytosol, rather than a low glycolytic rate followed by pyruvate oxidation. This increased glycolytic state causes a corresponding increase in cytosolic free NADH production. Increased NADH production in cancer cells is also the result of a heightened metabolic demand due to rapid cell division. Thus, NADH levels are indicative of cancer cell metabolic state. Specifically, a higher free/bound NADH ratio represents higher glycolytic activity, whereas a lower free/bound NADH ratio represents higher mitochondrial respiration. Without knowledge of NADH state (free or bound), NADH levels cannot be effectively studied. However, little is known about NADH state or distribution in cancer cells. Phasor-Fluorescence Lifetime Imaging Microscopy (Phasor-FLIM) is a useful tool to study NADH state and distribution, but NADH state and distribution are variable from cell to cell. NADH state analysis at different cell-cycle stages will allow for more specific and detailed comparisons between cancer cells. Here, Phasor-FLIM is used to quantify changes in NADH state and distribution during cell cycle progression. Different cancer cell lines, including Mb231 and U2OS, are used. Cancer cells in their flat, adherent interphase stage exhibit a low free/bound NADH ratio. During their rounded mitotic stage, cancer cells transition to a high free/bound NADH ratio and then return to a low free/bound NADH ratio as they divide. Therefore, striking change in NADH state during part of mitosis is observed. This suggests that cancer cells are at a high mitochondrial respiratory state in other stages but exhibit a spike in glycolysis during mitosis, returning to their original state by the time of division. The mitotic stages at which the spike occurs will be determined using mitosis specific fluorescent markers, and measurements will be compared to those in noncancerous cells.