

# Laser Scanning Cytometry for Assessment of DNA Damage

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*Dr. Darzynkiewicz's research focuses on the regulatory mechanisms associated with cell proliferation, apoptosis and sensitivity to anti-tumor drugs. He developed several techniques to analyze cell cycle kinetics, tumor progression, DNA damage and apoptosis that have world-wide application. He is the past President of the Cell Kinetics Society and the International Society for Advancement of Cytometry (ISAC), Fellow of the American Institute for Medical and Biological Engineering (AIMBE), Foreign Member of the Polish Academy of Sciences (PAN) and editor, co-editor or a member of the editorial board of 20 scientific journals. He has published over 670 papers which have been cited over 25,000 times, and has edited or co-authored 15 books.*

## Abstract

In live cells, DNA undergoes continuous damage caused by endogenous oxidants and environmental genotoxins. Because DNA repair, especially by non-homologous end-joining mechanisms, is error-prone, DNA repair errors accumulate. This contributes to cell senescence and preconditions to neoplasia. Most anticancer drugs target DNA, and their effectiveness relies on the degree of DNA damage that they induce in cancer cells. The assessment of DNA damage is thus of great importance in many disciplines of biology and medicine.

The sensitive reporters of DNA damage response are activation of ATM (A-ATM) through Ser1981 phosphorylation, phosphorylation of histone H2AX (P-H2AX) on Ser139, activation of Chk2 through Thr68 phosphorylation (A-Chk2) and phosphorylation of tumor suppressor p53 on Ser15. We have used phospho-specific antibodies to these proteins combined with multiparameter cytometry to correlate their phosphorylation with activation of caspase-3 (A-C3) and cell-cycle position, the events reporting induction of apoptosis and cell-cycle effects, respectively. Laser scanning cytometry (LSC) was particularly useful for evaluating the extent of DNA damage that involved formation of DNA double-strand breaks.

This multiplexed approach was applied to measure DNA damage induced by several exogenous genotoxins and metabolically generated oxidants. Exposure of cells to UV-B induced P-H2AX concomitant with A-C3, selectively in early-S phase and concurrent with activation of ATR and ATM. The DNA topo1-inhibitor topotecan triggered A-ATM, P-H2AX, A-Chk2 and A-C3 only in S-phase cells, with maximum at mid-S. DNA topo2-inhibitors mitoxantrone and etoposide induced A-ATM, P-H2AX and A-Chk2 in all phases of the cycle, with maximum in G<sub>1</sub>; A-C3, however, was seen in S-phase cells only. Replication stress caused by aphidicolin, hydroxyurea or thymidine led to P-H2AX and A-C3 (in S-phase) but did not trigger A-ATM. The genotoxins from tobacco smoke caused A-ATM, P-H2AX and A-Chk2

primarily in S-phase cells.

Constitutive A-ATM and P-H2AX were seen in untreated normal or tumor cells, reflecting the ongoing DNA damage caused by metabolically generated oxidants. The level of constitutive A-ATM and P-H2AX correlated with the cells' metabolic rate and was many-fold higher in mitogenically stimulated- than in G<sub>0</sub>-lymphocytes. The extent of constitutive A-ATM and P-H2AX, which was maximal during S and G<sub>2</sub>, was reduced by antioxidants and ROS scavengers (vitamin C, N-acetyl-cysteine, COX-2 inhibitors), metabolic inhibitors (2-deoxyglucose, 3-bromopyruvate) and growth at hypoxia. Analysis of constitutive A-ATM and P-H2AX provides the sensitive means to measure effectiveness of agents such as antioxidants or caloric restriction mimetics which, by neutralizing radicals or lowering aerobic metabolism, protect DNA from damage.

The use of multiparameter high-resolution cytometry to concurrently measure A-ATM, P-H2AX, A-Chk2, A-C3 and DNA content revealed a wealth of information on the associations among DNA damage, recruitment of DNA repair machinery, activation of cell-cycle checkpoints and induction of apoptosis. The capability of LSC to enumerate immunofluorescent (IF) foci of P-H2AX, A-ATM and A-Chk2, as well to measure maximal pixel in addition to the integrated fluorescence over cell nuclei, provided a highly sensitive instrumentation tool for analyzing constitutive DNA damage by endogenous oxidants. A novel approach utilizing LSC was also developed to quantitatively assess the depth of cell senescence.

#### **References:**

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