

## Assessment of DNA Damage Response by Cytometry

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### Abstract

DNA in live cells undergoes continuous damage caused by endogenously generated oxidants and by environmental genotoxins. Because repair of damaged DNA is error-prone, the defects accumulate with age, contributing to cell senescence and preconditioning to neoplasia. Effectiveness of most anticancer drugs relies on the extent of DNA damage that they induce in cancer cells. Assessment of the extent of DNA damage is thus of great importance in many disciplines of biology and medicine.

The sensitive reporters of DNA damage 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 in 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, concurrent with activation of ATR and ATM. 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 and they reflected the

ongoing DNA damage caused by metabolically generated oxidants. The level of constitutive A-ATM and P-H2AX correlated with cells' metabolic rates 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 anti-oxidants or caloric restriction mimetics which, through neutralizing radicals or lowering aerobic metabolism, protect DNA from damage.

The multiparameter high-resolution cytometry used to concurrently measure A-ATM, P-H2AX, A-Chk2, A-C3 and DNA content revealed a wealth of information on the association between 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 in analysis of constitutive DNA damage by endogenous oxidants.

#### **References:**

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