Abstract

Possibilities of flow cytometry in analysis of cellular response to genotoxic stress

Flow cytometry is a modern tool for interrogating the immunophenotype and functional characteristics of cells. It is a technique of quantitative single cell analysis that works by sensing optical properties of cells in a flow stream with laser beam. Analysis and differentiation of the cells is based on size, granularity, and whether the cell is carrying fluorescent molecules in the form of either conjugated antibodies or dyes. The cells may be stained with fluorescent dyes, incubated with fluorogenic substrate or labelled with fluorochrome-linked antibodies specific for molecules either on the surface or in the intracellular components of the cell. Flow cytometers are multiparameter, recording several measurements on each cell. Therefore, it is possible to identify a homogeneous subpopulation within a heterogeneous population of cells.

When cells are exposed to genotoxic agent, they respond quantitatively and qualitatively according to the absorbed dose and the cell type. To cope with the resulting damage to cellular DNA, the temporary cell-cycle checkpoints and DNA damage response mechanisms are activated to allow more time for effective repair. However, if these mechanisms fail or the damage is irreparable, then cell death via apoptosis or stress-induced premature senescence is induced. Among the different types of DNA damage, DNA double strand breaks (DSB) are the most detrimental type of DNA lesions. In molecular cell response to DSB, the conserved C-terminal tail of histone H2AX becomes rapidly phosphorylated at serine 139 by phosphatidylinositol 3-kinase (PI3K)-like kinases family, including ataxia telangiectasia mutated (ATM), ATR (ATM and Rad3-related protein), and DNA-dependent protein kinase (DNA-PK). The serine 139 phosphorylated histone H2AX is denoted as γH2AX, its creation peak approximately 1 hour after DSB formation and is one of the earliest known event involved in DNA damage response pathway.

The aim of our work was to quantify DSB *in vivo* and *in vitro*, cell cycle changes, apoptosis and senescence induced by genotoxic agents using selected flow cytometry methods. We used ionizing radiation, cisplatin, mitoxantrone and vanadocene dichloride as genotoxic agents. Experiments were performed using diverse prototype of cells: rat or human lymphocytes, mesenchymal stem cells, leukemia cell lines HL-60 and MOLT-4.

The immunofluorescence-based detection of γH2AX is a reliable and sensitive method for quantitatively measuring DNA DSB. Our findings indicate that quantification of γH2AX formation in rat peripheral blood lymphocytes 1 hour from irradiation make an early screening biological dosimeter for analyzing received dose of whole-body or thoracic radiation. This tool might help clinicians estimate received dose of radiation by organism and in consequence based on the results provide appropriate types of medical intervention to irradiated persons for optimal individual outcome. An increase in formation of γH2AX in rat peripheral blood lymphocytes was accompanied by dose dependent leukopenia and lymphopenia 24 hours following whole-body and thoracic irradiation of rat.

Mesenchymal stem cells respond to ionizing radiation by stress-induced premature senescence without induction of apotosis, even after high doses of gamma radiation. Quickly following irradiation DSB-induced γH2AX foci are formed in mesenchymal stem cells. Majority of DSB lesions are repaired within 24 h when most γH2AX foci have disappeared. The phosphorylation of H2AX was found to be ATM dependent. The inhibition of ATM by KU55933 completely abrogates the formation of γH2AX foci. Using cell cycle analysis we found that mesenchymal stem cells are preferentially accumulated in the G2 phase of the cell cycle in response to gamma irradiation. We observed increase in senescence-associated β-galactosidase activity using fluorogenic substrate and cytochemistry.

Our findings showed cytotoxic effect of vanadocene dichloride on leukemia cells, but unfortunately on human peripheral blood lymphocytes as well. Vanadocene dichloride induces apoptosis in leukemia cells; the induction is, however, lower than that of cisplatin. A linear dose-response relationship between vanadocene dichloride dose and phosphorylation of H2AX in leukemia cells 1 hour after vanadocene dichloride treatment was observed. The data indicate that cytotoxic effect of vanadocene dichloride on leukemia cells can be particularly mediated by the formation of DSB.