
Measurements of the DNA double-strand break response

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av

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- I. Muslimović A, Nyström S, Gao Y, Hammarsten O. Numerical analysis of etoposide induced DNA breaks. PLoS ONE 2009 4(6):e5859.
- II. Muslimović A, Ismail IH, Gao Y, Hammarsten O. An optimized method for measurement of gamma-H2AX in blood mononuclear and cultured cells. Nat Protocols 2008 3(7):1187-93.
- III. Muslimović A, Johansson P, Ruetschi U, Hammarsten O. Calibrators for clinical measurements of phosphorylated H2AX in patient cells by flow cytometry. Submitted Manuscript.
- IV. Johansson P, Muslimović A, Hultborn R, Fernström E, Hammarsten O. In-solution staining and arraying method from the immunofluorescence detection of γ H2AX foci optimized for clinical applications. Biotechniques 2011 51(3):185-9



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Abstract

Measurements of the DNA double-strand break response

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Radiotherapy and some chemotherapeutic drugs kill cancer cells by induction of the extremely toxic DNA double-strand breaks (DSBs). Measurements of the DSB response in patients during therapy could allow personalized dosing to improve tumor response and minimize side effects. DSBs induce a strong cellular response via phosphorylation of H2AX, P-H2AX and the formation of foci. P-H2AX can be measured by flow cytometry or counted in separate cell nuclei by immunofluorescence microscopy. The overall aim of my research has been to develop and validate methods to measure P-H2AX in mononuclear cells from cancer patients undergoing radiotherapy.

Initially, we wanted to characterize DNA damage induced by the chemotherapeutic drug etoposide that induces TopoII-linked DSBs and to test its ability to activate the P-H2AX response using a flow cytometry-based P-H2AX assay. We found that only 0.3% of etoposide-induced DSBs activated the H2AX response and toxicity. We concluded that the P-H2AX response was a good measure of the toxic effects of etoposide.

Next, we wanted to optimize the flow cytometry assay for mononuclear cells from cancer patients undergoing radiation therapy. The P-H2AX response was measured before and after 5Gy pelvic irradiation and in *in vitro*-irradiated controls. We found a fraction of cells with high P-H2AX signals that corresponded to the 5Gy *in vitro*-irradiated blood controls. This study indicated that flow cytometry may be well suited for measurements of the P-H2AX response in mononuclear cells following local radiotherapy.

To be able to implement the P-H2AX assay in clinical practice and use it in relation to clinical outcome and side effects we have also developed stable and reliable calibrators based on phosphopeptide-coated beads and fixed cells. Using these calibrators it could be possible to use the P-H2AX flow cytometry assay in the clinic in a controlled manner.

Finally, using immunostaining in solution before cells are mounted on microscopic slides for quantification of single P-H2AX foci by immunofluorescence, we have the possibility to analyze 16 patient samples within few hours, which makes this method suitable for clinical use.

Keywords: *DNA double-strand break, DSB; phosphorylated H2AX, P-H2AX; etoposide; ionizing radiation, IR; flow cytometry; calibrators; immunofluorescence; P-H2AX foci.*

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