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Review

γH2AX as a marker of DNA double strand breaks and genomic instability in human population studies

Vanessa Valdiglesias [a,1], Simona Giunta [b,1,2], Michael Fenech [b], Monica Neri [a], Stefano Bonassi [a,*]

[a] Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Roma, Italy
[b] CSIRO Preventative Health Flagship, Adelaide 5000, Australia

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ABSTRACT

DNA double strand breaks (DSB) are the gravest form of DNA damage in eukaryotic cells. Failure to detect DSB and activate appropriate DNA damage responses can cause genomic instability, leading to tumorigenesis and possibly accelerated aging. Phosphorylated histone H2AX (γH2AX) is used as a biomarker of cellular response to DSB and its potential for monitoring DNA damage and repair in human populations has been explored in this review. A systematic search was conducted in PubMed for articles, in English, on human studies reporting γH2AX as a biomarker of either DNA repair or DNA damage. A total of 68 publications were identified. Thirty-four studies (50.0%) evaluated the effect of medical procedures or treatments on γH2AX levels; 20 (29.4%) monitored γH2AX in specific pathological conditions with a case/control or case/case design; 5 studies (7.4%) evaluated the effect of environmental genotoxic exposures, and 9 (13.2%) were descriptive studies on cancer and aging. Peripheral blood lymphocytes (44.6%) or biopsies/tissue specimens (24.3%) were the most commonly used samples. γH2AX was scored by optical microscopy as immunostained foci (78%), or by flow cytometry (16%). Critical features affecting the reliability of the assay, including protocols heterogeneity, specimen, cell cycle, kinetics, study design, and statistical analysis, are hereby discussed. Because of its sensitivity, efficiency and mechanistic relevance, the γH2AX assay has great potential as a DNA damage biomarker; however, the technical and epidemiological heterogeneity highlighted in this review infer a necessity for experimental standardization of the assay.

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Contents

* Corresponding author at: Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Via di Val Cannuta, 247, 00166 Rome, Italy. Tel.: +39 065 225 3418; fax: +39 065 225 5668.
E-mail address: stefano.bonassi@sanraffaele.it (S. Bonassi).
[1] These authors contributed equally to this work.
[2] Current address: The Rockefeller University, New York, NY 10065, USA.

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1. Introduction: γH2AX as a hallmark of DNA double strand breaks

On a daily basis, genetic material in the cell suffers a wide variety of lesions, caused by exogenous and endogenous sources. Double strand breaks (DSB) are breaks that encompass both strands of the DNA helix and are the gravest form of damage for eukaryotic cells [1,2]. DSB occur following exposure to ionizing radiations (IR), genotoxic chemicals or radiomimetic drugs, including cancer chemotherapeutics. Failure to repair DSB, or their misrepair, can cause genomic instability. Alterations in gene dosage or generation of chromosomal abnormalities may lead to tumorigenesis and other age-related diseases (reviewed in [3,4]).

The H2A core histone variant H2AX plays an important role in the DNA damage response following induction of DSB. H2AX constitutes approximately 10% of nucleosomal H2A in human cells [5], occurring roughly once every ten nucleosomes, mostly depending on cell types. Following the generation of DSB, H2AX histones located near the DNA break are quickly phosphorylated by members of the phosphatidyl-inositol-3-kinase-related kinases (PIKKs) family. This phosphorylated form of histone H2AX is referred to as γH2AX and is a marker of DNA damage. γH2AX accumulates within seconds following induction of a DSB and spreads over tens of kilobases of DNA flanking the break site, playing a role in the amplification of the DNA damage responses (DDR) signaling cascade [6,7] (Fig. 1). Cells lacking H2AX repair more slowly and display gross chromosomal rearrangements compared to control cells, indicating a role for γH2AX in maintenance of genomic stability [6].

γH2AX forms nuclear domains, termed DNA damage foci, that can be cytologically visualized by fluorescent microscopy [5]. Importantly, the number of foci formed per cell nucleus reflects the number of induced DSB [8–10], suggesting that each γH2AX focus corresponds to a DSB. Phosphorylation of H2AX can be experimentally induced by treatment with IR, radiomimetic chemicals, and use of restriction endonucleases [11–13].

Several methods exist to detect and quantify DSB, such as pulse-field gel electrophoresis (PFGE) and neutral comet assay; however, the γH2AX assay offers some key experimental advantages, being technically simpler, precise and more sensitive than other techniques, detecting presence of a single DSB. The fluorescent immunohistochemical visualization of γH2AX foci is a versatile approach which allows both quantification and localization of DSB.

Phosphorylation of H2AX in response to DNA damage has been observed in quiescent and cycling cells, and during all phases of the cell cycle, including mitosis [5,14,15]. γH2AX has also been observed in human pre-cancerous lesions [16], and in a wide variety of tumors, including melanoma [17], colon carcinoma, osteosarcoma, fibrosarcoma [18] and cervical cancer [19]. Thus, in addition to serving as a specific marker for genotoxic damage, γH2AX might be utilized for early cancer screenings and is involved in apoptosis and senescence pathways [20,21]. It is noteworthy, however, that γH2AX staining, can be induced by cellular stress, heat [22,23] synchronizing agents, such as the microtubule-depolimerizing drug nocodazole [14], as well as by other forms of DNA damage, including UV, and cellular processes like apoptosis [24,25]; however, these staining patterns are often morphologically different from the punctuate staining observed upon DSB induction.

In this review, we will explore for the first time the potential of the γH2AX assay for human population studies. A systematic evaluation of published population studies employing the γH2AX assay as a marker of DNA damage and repair will be presented. The different exposures investigated, the strength and weaknesses of the study design, the effect of technical heterogeneity, the concordance with other biomarkers, and the perspectives for a wider application of this assay will be extensively evaluated and discussed. This review also aims to highlight standardization steps that will have to be implemented to make the γH2AX assay a widespread and reliable tool for human population studies.

2. Literature review of epidemiological studies employing the γH2AX assay

Since the discovery of phosphorylation of H2AX following DSB formation, the γH2AX assay has been extensively applied in fundamental and basic research. More recently, the application of this assay in human population studies has been reported. The specificity in recognizing DSB and sensitivity in allowing detection of low number of DSB, the short time frame of the protocol, the small quantities of biological sample required, and the possibility to perform automated scoring, supports its use as a biomarker of genomic instability and genotoxicity, as demonstrated by the increasing number of epidemiological studies using this assay.

2.1. Methods

2.1.1. Bibliographic search

The identification and selection of epidemiological studies to be included in this review was carried out through an extensive literature search using the PubMed database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA; http://www.ncbi.nih.gov/PubMed), and was updated to July, 1st 2012.

The search strategy was the following: "H2AX protein, human"[Supplementary Concept] OR gammaH2AX[tiab] OR H2AX[tiab] AND (English[lang] AND (("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]) OR "adult"[MeSH Terms])).

One hundred and fifty-six citations were obtained and manually reviewed. Among them, 60 studies fulfilled the selection criteria listed in Section 2.1.2 and were included in the systematic review, together with 8 additional publications that were identified from the references of published articles.

2.1.2. Selection criteria

Eligible studies included in the review were those conducted in humans, written in English, reporting γH2AX levels as a biomarker of either DNA damage or DNA repair (*i.e.* after *in vitro* irradiation), and obtained with any of these different methods: immunofluorescence, flow cytometry or immunoblotting. Studies in blood cells or other surrogate cells, cancer tissue, biopsies or surgical specimens were included, while those in established cell lines

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