Can *γH2AX* assay be an endpoint in drug discovery?

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Biomarker development is essential in the drug discovery field for effectiveness, toxicity, and optimization of doses when a drug is translated during pre-clinical to clinical trials. The present review highlights the prospective application of the *γH2AX* assay for pharmacological and toxicological evaluation in drug development. *γH2AX* is a phosphorylated form of H2AX protein belongs to the family H2A and is a well-known biomarker for measurement of DNA double strand breaks (DSBs). During chemotherapy, cancer cells are targeted by drugs that induce DSBs thereby result in cell death. In such scenario, *γH2AX* could be an informative marker for the effectiveness of the chemotherapeutic drug as wells as its associated toxicity in surrounding normal cells. Moreover, the canonical calculation of dose conversion of a drug from pre-clinical animal models to clinical trials in humans or non-human primates may not be effective all the time. The *γH2AX* assay may be used as one of possible marker for such dose conversion for any drug capable to generate DSBs. Besides, it can also be used as an effective biomarker in radiation countermeasures and bio-dosimeter which has been substantiated in many research articles. Considering the importance of *γH2AX* in various fields, this biomarker could be helpful in safety and efficacy assessment of new drugs.

Key Words: *γH2AX*, Clinical pharmacology, toxicity

INTRODUCTION

Drug discovery is an excellent area of research which needs vast and latest updated knowledge for the development of any new drug for human use. So far, a tremendous progress has been made in formulating guidelines required during various phases of drug development. Pharmacological mechanism of action integrated with safety evaluation play a paramount importance for drug discovery. Enormous biological parameters are being evaluated for understanding the mechanism of action for an agent. A number of bioassays have been put to use for safety, efficacy and mechanistic aspects as per the guidelines of the various drug approving agencies operating across the world. These bioassays serve as an essential tool for analyzing the pharmacological mechanism of an agent. The search for a suitable biomarker is an ongoing process which requires a pragmatic approach based on various ways of experimentation.

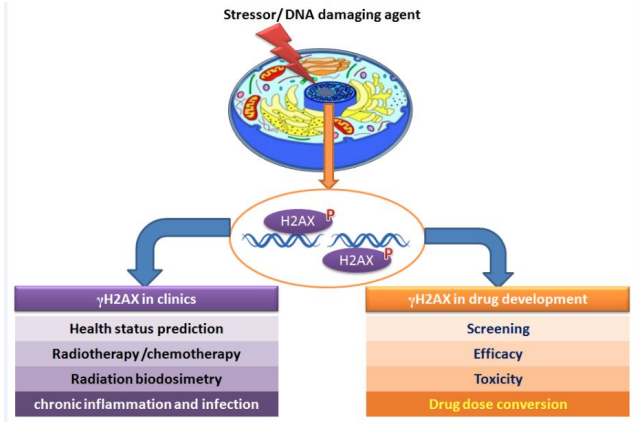

Figure 1: *γH2AX* as a biomarker in clinics and in drug discovery.

From past twenty years, since the discovery of *γH2AX*, this marker has been used vastly by various researches to understand the mechanistic aspects of DNA double strand breaks (DSBs) response pathway. However, the use of this protein in applied research is still needed to be explored. The present review potentiates application of *γH2AX* as a biomarker in clinics and in drug discovery field **(Figure 1).**

γH2AX Genesis

Any stress agent that generates DSB leads to an extensive response in the chromatin region flanking the break. A large number of protein species accumulates at DSB sites forming large nuclear aggregates [1]. Ser-139 phosphorylated form of H2AX (*γH2AX*) protein is a unique biomarker of DNA double strand break. In 1998, Rogakou et al., first time discovered this variant of histone protein in mammalian systems exposed to an ionizing radiation [2]. H2AX is a histone H2A variant which constitutes 2-25% of histone H2A [2,3] and is composed of a central globular domain, flanked by N-terminal and a unique COOH terminal prone to post-translational modifications like acetylation, biotinylation, phosphorylation, methylation, and ubiquitination [2,4]. The proteins responsible for the phosphorylation of the H2AX protein are ataxia telangiectasia mutated (ATM), AT and Rad3-related protein (ATR), and DNA-dependent protein kinase (DNA-PK) [5]. However, Peng et al. in 2008, suggested its auto-phosphorylation ability [6].

Kinetics of *γH2AX*

The persistence of phosphorylated form of H2AX in the cellular system is debatable that it persisted for shorter or longer duration [7]. Previous reports convey the formation of *γH2AX* within a minute which generally persists up to 24-48 h [8-10]. However, recently many research articles have shown its persistence for days or even months in various model systems [7,11-13]. The short existence of this marker in cells can allow quick estimation, however, posing a great limitation for evaluation of DNA damage for longer time points. Moreover, the persistence of this protein for many days indicates the residual DNA damage that could be used as a marker for assessment of manifestation of prolonged damage.

γH2AX in clinics

γH2AX can play a major role in clinical settings viz-a-viz diagnosis and mortality/morbidity prediction of radiation-exposed individuals. Apart from this it has also wider applications in radiation bio-dosimetry

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assessment. Moreover, *γH2AX* can also help in the prediction for a type of radiation exposure and in treatment of chronic inflammation/infection.

γH2AX in diagnosis

At instances DSBs may leads to cancer however, paradoxically; this also helps in killing cancerous cells. *γH2AX*, which is generated upon DSBs generation, could play an important role in diagnosis and effectiveness of treatment in patients suffering from carcinoma [14]. Worldwide, the major accepted methods used for the treatment of cancer are radiotherapy and chemotherapy [15]. Even after the advancement in these treatments modalities for treatment of tumor, very limited success has been achieved in this direction. The probable reasons are ineffectiveness and associate toxicity with almost all the treatment protocols in addition to contributory factors responsible for tumor formation. Basically, these two treatment protocols kills the cancerous cells by generating the DSBs [16]. So, the biomarker, *γH2AX*, has been measured in various clinical cases involving the patient for evaluation of DNA damage making a strong reasoning to be used as a marker for determining efficacy of treatment protocol, dose standardization/optimization and associated toxicity in surrounding tissues [17-19]. Taking this into consideration *γH2AX* has been studied against variety of cancer types, including the adrenal renal cancer [20], head& neck cancer [21], cervix cancer [22], breast cancer [23], rectal cancer [24], lung cancer [25], and ovary cancers [26].

γH2AX in prediction of mortality/morbidity of patents

γH2AX could be a marker for prediction of mortality/ morbidity in patients undergoing clinical treatments for radiation or chemical or some chronic diseases. In a case study published by Gupta et al, 2013, they have reported the detection of *γH2AX* after a month in metal scrap workers accidentally exposed to Co60 radiation [11]. This indicated the persistence of *γH2AX* form for longer period. Moreover, every patient has shown variable level of *γH2AX*. The patient who exhibited a highly significant level *γH2AX* consequently died later which clearly indicate the role of this protein in predicting severity, in terms of overall health status, of an individual [11]. In another study published by Yashavarddhan et al., confirmed these observations in rabbit model system [7]. In this study, they have explained *γH2AX* marker in studying the severity of radiation exposure and morbidity status of animals. Besides extensive radiation related studies, there are few reports showing a presence of this biomarker in chronic obstructive pulmonary disease (COPD) and tumorigenesis [4,27,28]. Activation of the proto-oncogene in chronic inflammation leads the development of cancer [29,30]. Their activations affect the cell cycle checkpoints that lead to the production of reactive oxygen species which damage the DNA. This damage can be measured by *γH2AX*. COPD is one of the examples of chronic inflammation in which few research articles have shown the measurement of *γH2AX* [29]. Viral infections targeting the DNA may also be addressed by the use of this biomarker [30]. Considering all the available literature, *γH2AX* may be a potential biomarker for the assessment of mortality, morbidity, and health status of individuals exposed to genotoxic agents.

Radiation biodosimetry and prediction of type of radiation exposure

Extensive data are available for estimation of *γH2AX* in radiation biodosimetry. The linear response of this protein with radiation doses prompted various investigators to use it as a biomarker for radiation biodosimetry [31-33]. The gold standard marker for quantification of radiation damage is dicentric assay which is labor intensive and needs skills as well as longer time for delivery of the result [34]. However, the formation of *γH2AX* within a minute of the exposure and its measurement using foci measurement or through flow-cytometric techniques makes it a very useful biomarker for dose assessment. The quick estimation of dose in radiation-exposed patient could certainly help the clinician to decide the treatment regimen. The interlaboratory evaluations are undergoing to validate this marker for radiation dose assessment. Additionally, the *γH2AX* may also predict the type of radiation exposure such as acute or repeated. The findings have shown larger *γH2AX* foci size and longer persistence for repeated radiation exposure compared to acute exposure [7,11]. However, further validation of these reports by other investigators could support this view.

γH2AX in pharmaceutical development

Drug development is a tedious process that needs lot of time and efforts for the translation of a drug molecule from bench to market. Chemotherapeutic drugs have shown the DNA damage induction in cells which can be evidenced by *γH2AX* assay [35]. Olive and Banath in 2009 used this assay to measure the effectiveness of Cisplatin, which is a worldwide used chemotherapy drug, in cancerous cells [36]. The similar experimental approach was adopted by various researchers to measure the effectiveness of various chemotherapy drugs like gemcitabine, busulfan, and melphalan, against a different type of cancer [37]. Additionally, this maker has been recently used for evaluation of the radioprotective efficacy of radiation countermeasure agents which were in pre-clinical stage [7,10,38,39].

γH2AX in toxicity studies

Toxicity measurement of a drug is a major parameter for its development. An ideal drug should be nontoxic or limited undesirable effect. OECD guidelines for toxicity show various standard assays for evaluation of toxicity of any compound. Genotoxicity studies generally involve micronuclei, comet, and chromosomal assays. Researchers have also used *γH2AX* assay for evaluation of toxicity [40]. The method for measurement of *γH2AX* requires minimum time and very quick, so it may be used as supportive parameters for estimation of genotoxicity.

γH2AX in drug dose conversion

From last few decades dose conversion from lower animals to higher animals applies the classical conversion formula [41] which may not be effective for many reasons. There is need to develop reliable assays which can support in dose conversion. Considering the available literature the *γH2AX* assay could be helpful in deciding such dose conversion particularly for the drug inducing DNA DSBs.

CONCLUSION

The entire above summary about *γH2AX* clearly shows its relevance in a different domain of drug discovery along with its usefulness in clinics. The characteristic formation of this protein in the cells exposed to any stress and resulted in DSBs has made it a marker for evaluation of toxicity, efficacy and mechanism study. Commendable publication in last few decades indicates its immense potential as a biomarker in radiation biodosimetry. Henceforth, the researcher should take advantage of this marker in various fields of clinical therapeutics, diagnosis and in drug discovery for efficient and quick evaluation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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