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γ-H2AX as a protein biomarker for radiation exposure response in ductal carcinoma breast tumors: Experimental evidence and literature review

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► ***Review article***

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ABSTRACT

Background: H2AX is a histone variant that is systematically found and ubiquitously distributed throughout the genome. DNA double-strand breaks (DSBs) induce phosphorylation of H2AX at serine 139 (γH2AX), an immunocytochemical assay with antibodies recognizing γH2AX has become the gold standard for the detection of DSBs. The importance of this assay to investigate different individual responses to gamma irradiation was reviewed and an example of different radiation responses of ductal carcinoma tumors with different expression levels of ATM and HER-2 was discussed. ***Materials and Methods:*** The ductal carcinoma breast tissues were exposed to 4 Gy gamma rays and after 24 hours incubation in modified RPMI 1640 medium in 37 °C with CO_2, the frequency of residual induced DSB was assessed using γH2AX assay compared to pair normal adjacent and control breast tissues. ***Results:*** Results showed that the frequency of DSB dramatically increased in both tumor and normal irradiated tissues, compared to sham non-irradiated controls. Tumors with HER-2 over expression showed significantly lower residual DSB frequencies after 24 hours post irradiation incubation time, whereas this frequency dramatically increased in ATM under expressed tissues. ***Conclusion:*** Our data showed that different tissues may have different radio-sensitivity and ATM under- and HER-2 over-expression may lead to higher and lower sensitivity to ionizing radiation, respectively. This may be due to the role of ATM in DSB repair and HER-2 in EGFR downstream signaling pathway that with the use of cell survival mechanisms ends to resistance against radiation effects and activation of PI3K/ACT that leads to DSB repair.

Keywords: *γ-H2AX, radiation response, DSB, HER-2, ATM, ductal carcinoma.*

INTRODUCTION

Radiotherapy is an effective cancer treatment, but, there are individualized variable responses and also even with highly conformal treatment planning, associated radiation toxicities in neighboring normal tissues remain the major limiting factor for delivering tumoricidal doses. Clear differences exist between patients regarding their individual tissue responses after radiotherapy. Even after strictly identical treatment modalities, some patients seem to have different responses even in their normal tissues; some tolerate the treatment well, whereas others develop severe radiation induced side effects. There is increasing evidence that the patient-to-patient variability in tissue response is caused primarily by their genetic predisposition, by subtle mutations, or polymorphisms in genes involved in cellular responses to radiation [1-3]. The study of damage to cellular DNA is essential for the understanding of cell apoptosis and mutation that may lead to serious disease including

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cancer. Changes in the genome caused by a single double strand break (DSB) may be enough to induce cancer or cell death [4]. A mechanism by which DSBs can be located within the nucleus and quantified is highly desirable. Predictive assays that accurately determine normal tissue tolerance in individual patients would permit to modify the treatment in radiosensitive individuals to prevent severe side effects and to intensify radiotherapy in relatively resistant patients, thereby improving the therapeutic ratio in cancer treatment [5-7]. Because the DSB is the critical lesion induced by ionizing radiation in terms of cell killing, their analysis provides essential insight into fundamental and translational radiobiology.

However, DSBs are relatively infrequent as compared to the other radiation-induced lesions such as SSB and base damage, resulting in technical challenges in the development of specific analytical procedures. Standard techniques for quantifying DSB induction and repair have included pulsed field gel electrophoresis (PFGE) and the neutral comet assay [8]. Over the last several years, γ- H2AX expression has been established as a sensitive indicator of DSBs [9]. Although the specific role of γ-H2AX in the repair of DSBs has not been defined, recent reports indicate that the dephosphoryation of γ- H2AX and dispersal of γ -H2AX foci in irradiated cells correlates with the repair of DNA DSBs [10-12]. H2AX is a histone variant that is systematically found and ubiquitously distributed throughout the genome the biological functions of H2AX are as follows: - concentration of DNA damage signaling and repair proteins at DSBs [13]; - signal amplification and transduction to enhance the sensitivity of the DNA damage induced G2 cell cycle checkpoint [14]; - implementation of an Artemis-dependent pathway required for the processing of a subset of radiation-induced DSBs; - recruitment of cohesion to promote sister chromatid- dependent recombinational repair [15]; - chromatin remodeling to assist DSB processing [16]; - a chromatin anchor to prevent dissociation of break ends and enhance repair fidelity [17-18].

At sites of radiation-induced DNA DSBs, the histone H2AX becomes rapidly phosphorylated (the phosphorylated form is referred to as γ-H2AX) forming readily visible nuclear foci [19,20]. We can make use of the cell's natural protein function targeted at the repair of such breaks to reveal break locations under fluorescence microscopy.

Phosphorylation of the chromatin protein H2AX (forming γH2AX) is implicated in the DSB repair pathway; a large number of H2AX molecules become phosphorylated at the sites of DSB's. Fluorescent staining of the cell nuclei for γH2AX, via an antibody, enables us to visualize the formation of these foci, allowing the quantification of DNA DSB's and forming the basis for a sensitive biological dosimeter of ionizing radiation. There is a one to one correspondence between the number of DSB's and γH2AX foci [20]. Counting and quantifying the γH2AX foci necessarily requires probing individual, intact cells.

To date, investigations of mechanisms that regulate H2AX phosphorylation have been conducted predominantly through two-dimensional (2D) immunofluorescence (IF) using antibodies that recognize the phospho-serine residue of γ-H2AX. In 1999, the laboratory of William M. Bonner published the first γ-H2AX 2D IF experiments, which revealed that H2AX phosphorylation could be visually monitored by accumulation of anti-γ-H2AX antibodies into sub-nuclear regions referred to as foci [21]. The authors demonstrated that 1) the numbers of γ-H2AX foci induced by ionizing radiation (IR) were comparable to the estimated numbers of IR-induced DSBs, and 2) γ-H2AX foci co-localized with the path of a laser capable of cleaving chromosomal DNA strands [21].

The scoring of foci is currently the most sensitive method for γ-H2AX analysis. A single DSB results in the phosphorylation of thousands of H2AX proteins over chromatin domains of several mega bases of DNA either side of the break. γ-H2AX foci become microscopically visible within minutes after irradiation, with an average early size of 0.2 μm2 indicating the rapid phosphorylation of thousands γ-H2AX molecules in domains of approximately 2 Mbp [22]. Thanks to this large scale formation of

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γ-H2AX, focused in a sub-micron volume, foci can be easily distinguished from a relatively homogeneous background signal so that one individual DSB can be detected.

The goal of this study was to study the responses of different human breast tissues with different expression levels of ATM and HER-2 to gamma irradiation using γ-H2AX assay.

MATERIALS AND METHODS

Subjects and study design

Eligible subjects were ten breast tissues of breast ductal carcinoma patients with different statues of ATM expression and HER-2 gene amplifications (HER-2 gene amplification and ATM expression statues were analyzed and reported in our previous studies [23,24]. We included ten normal adjacent breast tissues and five normal breast tissues retrieved from not affected woman undergone breast reduction surgery for beauty purposes as the control groups. The dissected tissues transferred to RPMI1640 complete medium (Gibco-BRL) immediately and underwent 4 Gy gamma irradiation using cobalt -60 (Theratron, 789-C Canada) then incubated in 37 °C with 5% CO_2 for 24 hours following preparation of 10% formalin fixed paraffin embedded blocks and slides with 4 micrometer tissue section thickness using standard methods.

γ H2AX-immunofluorescence assay

After de-waxing in xylene and rehydration in graded alcohols, sections on slides were boiled in citrate buffer and pre-incubated with fetal calf serum (FBS). Afterwards sections were incubated with anti- γ H2AX antibody (Upstate; 1:800), followed by FITC–conjugated Rabit-IgG secondary antibody (Invitrogen; 1:400). Finally, sections were mounted in VECTA shield with 4',6-diamidino-2-phenylindole. From the step using the secondary antibody onward, all procedures were performed in the dark.

Foci analysis

All slides were analyzed using a Nikon E800 Eclipse microscope (Nikon, Tokyo, Japan) equipped with epifluorescence and triple band pass filters. For quantitative analysis, foci were counted by eye using objective magnification of ×60 and ×100. Foci counting were done until 80 cells and 40 foci were registered for each data point. Cells were classified as positive (i.e., containing radiation-induced γ-H2AX foci) when more than five foci were detected.

Statistical analysis

Experiments were repeated thrice at each data point (HER-2 positive, HER-2 negative, normal ATM expression, ATM over expression, ATM under expression, normal adjacent and sham control groups) and statistical analysis was done using a non-parametric Kruskal-Wallis one-way analysis of variance test. Data are presented as mean of foci per cell and the frequency of cells with ≥5 foci. A probability level of a P value of <0.05 was considered significant.

RESULTS

To investigate the residual DSB induced by 4 Gy gamma rays after 24 hours as a repair time, γ H2AX assay was established. The study group was 28 breast tumors composed of 10 ductal carcinoma with / without HER-2 gene amplification each one in 5 and with different expression levels of ATM, 10 normal adjacent breast tissues of these patients, 5 breast tissues of women with no breast disease in them nor their family undergone surgery for breast reduction purposes mentioned as control group and finally sham control group consisted of 3 breast tissues each from one of previously mentioned test groups with no irradiation.

The results of analysis of residual DSBs are summarized in table 1 and shown in figure 1. As shown in figure 1, residual DSB manifesting as fluorescent foci was dramatically increased in all irradiated groups compared to non irradiated sham controls. Five HER-2 gene amplified ductal carcinoma tumors showed statistically significant less DSBs compared to the other ones.

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Table 1. results of residual induced DSBs analyzed using γ H2AX assay in ductal carcinoma breast tissues with / without HER-2 gene amplification and different expression status of ATM , normal tumor adjacent breast tissues and controls.

Samples	Mean of foci per cell	Frequency of cells with ≥ 5 foci	HER-2 situation	ATM expression
T1	4	12%	amplification	Normal
T2	3	10%	amplification	Normal
T3	2.5	10%	amplification	Normal
T4	3.5	12%	amplification	Normal
T5	4.2	14%	amplification	Normal
T6	6	30%	No amplification	Normal
T7	12	76%	No amplification	Under exp.
T8	7	50%	No amplification	Normal
T9	13.5	80%	No amplification	Under exp.
T10	14	92%	No amplification	Under exp.
N_{adj} 1	6.2	40%		
N_{adj}2	7	64%		
N_{adj}3	6.5	51%		
N_{adj}4	6	56%		
N_{adj}5	5.8	57%		
N_{adj}6	6.5	46%		
N_{adj}7	10	67%		
N_{adj}8	6.2	40%		
N_{adj}9	9.5	70%		
N_{adj}10	10	72%		
C1	6	30%		
C2	6.5	42%		
C3	5.8	32%		
C4	6.3	50%		
C5	7	70%		
Sham control T	0.5	0%		
Sham control N_{adj}.	o.6	0%		
Sham control C	0.12	0%		

T: tumor, N_{adj} : normal adjacent tissues of tumor, C: normal control breast tissue, Sham control: non irradiated tissue samples.

As shown in figure 1, the three samples with highest DSBs frequency in both tumor and normal adjacent tissues (sample 8-10) showed ATM under expression. To facilitate comparing the result of residual DSBs, the test tissues were studied in two distinct groups: one with HER-2 gene amplification and the other with normal gene amplification. Also ATM expression was analyzed in two groups with normal and under expressed situations.

As shown in figure 2 the frequency of residual DSBs in ductal carcinoma tumors with ATM under expression was significantly higher compared to other normal ATM expressed ductal carcinoma tumors, normal adjacent and control ones. Also the statistically significant difference was observed in frequency of DSBs in normal adjacent tissues of individuals with under expression of ATM compared to control groups. To confirm our findings, we did an independent analysis to evaluate the DSB repair in solid tissues. Instead of counting gH2AX-foci per cell, we quantified the number of cells with ≥5 γ H2AX-foci, as shown in figure 3; this alternative evaluation procedure provides similar results with regard to the DSB repair capacities of the different expression of ATM in tumor and normal tissues, which support our original conclusions.

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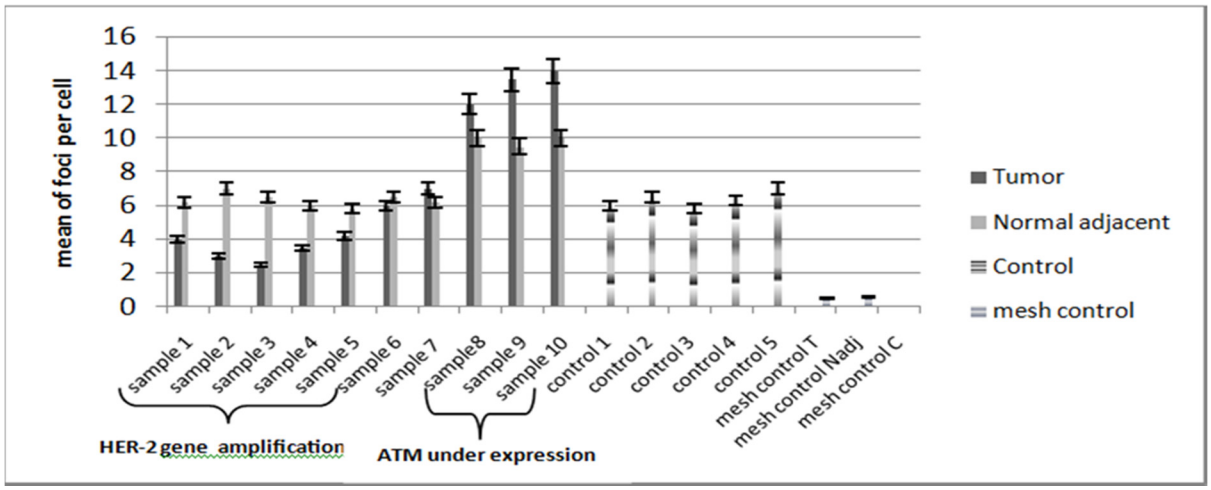


Figure 1. Mean of foci per cell in ten ductal carcinoma tumor and normal adjacent breast tissues compared to 5 controls after 4 Gy gamma irradiation followed by 24 hours repairing time compared to non irradiated sham controls.

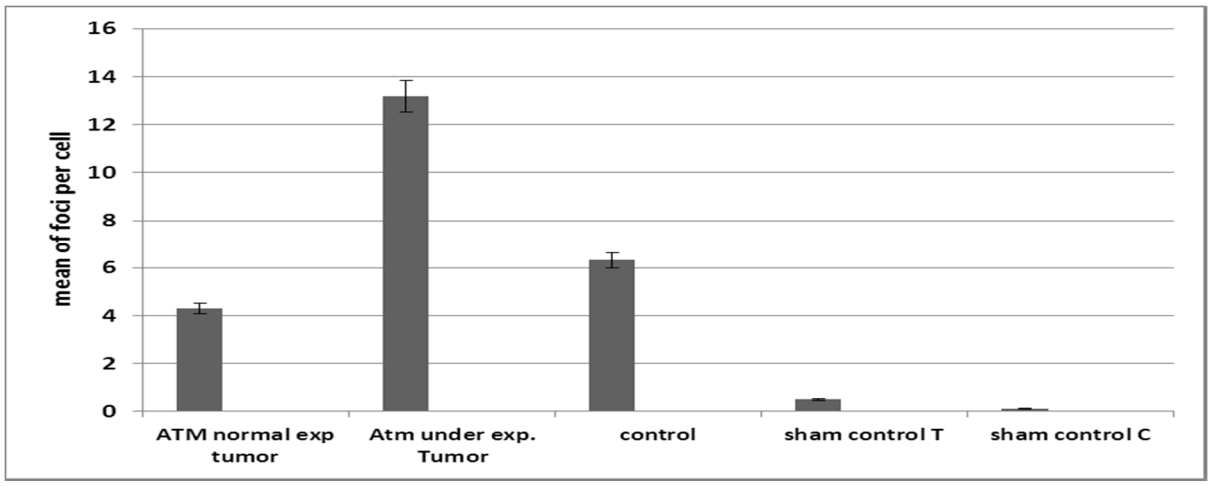


Figure 2. mean of foci manifesting residual DSBs in irradiated ductal carcinoma tumors with normal and under expression of ATM compared to control and sham non irradiated controls.

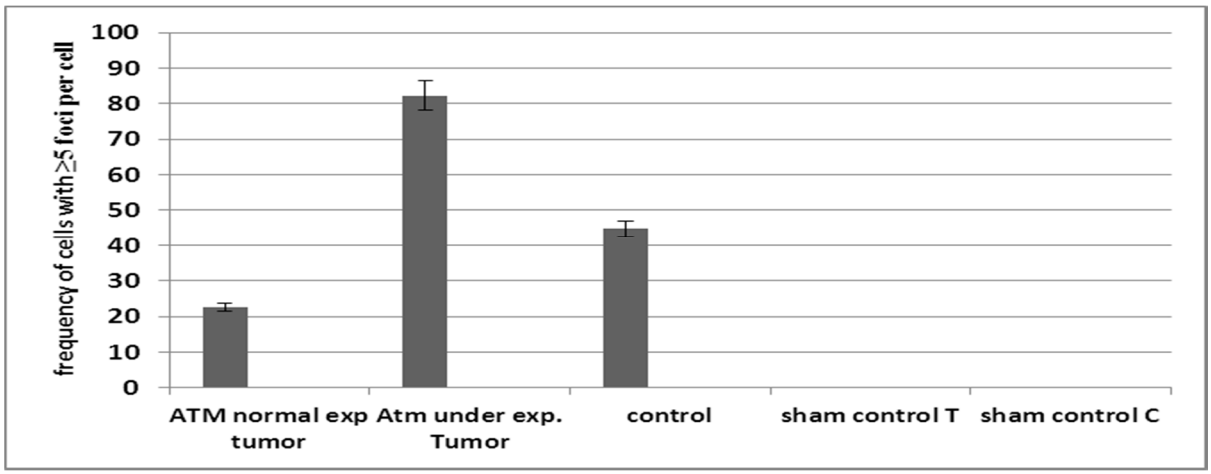


Figure 3. Frequency of cells with ≥5 foci per cell in 4 Gy gamma irradiated ductal carcinoma breast tumor tissues with normal and under expression of ATM compared to control after 24 hours repair time compared to non-irradiated sham control groups.

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As shown in figure 4, the frequency of residual DSB after 24 hours 4 Gy gamma irradiation significantly decreased in ductal carcinoma tumors with HER-2 gene amplification compared to tumors with normal gene amplification and control ($p< 0.01$).

As a coincidence in our study 3 of samples in non amplified HER-2 gene tumors had ATM under expression, since the frequency of induced DSB was dramatically increased in ATM under expressed tumors, caused the frequency of DSB in non HER-2 gene amplified group over estimated compared to HER-2 gene amplified ones and control. To avoid this frequency of residual induced DSB was analyzed in two ways, in the absence and the presence of those three ATM under expressed samples. As shown in figure 5, about 11.6% of cells with HER-2 gene amplified tumors had at least 5 foci whereas in normal amplification of HER-2 this frequency was about 69.4 in the presence of those three ATM under expressed samples and 50% in the absence of them. The frequency of cells with more than 5 foci per cell in control tissues was 44.8%.

DISCUSSION

In eukaryotes, DNA is highly condensed and packaged into chromatin within the nuclei. This condensed chromatin forms a structural barrier for DNA processing during DNA repair, replication, transcription, and recombination. A fundamental subunit of the chromosome is the nucleosome, which is composed of 146 bp of DNA wrapped in two complete turns around an octamer of the core histones H2A, H2B, H3, and H4, and there are varying lengths of linker DNA connecting these subunits. The core histone octamer forms a 100-kDa protein complex [25]. Histone H2A has been conserved throughout eukaryotic evolution. There are three H2A subfamilies: H2A1, H2A2, H2AZ, and H2AX [26]. Histone variants are non-allelic isoforms that replace major histones within the nucleosome. These proteins are classified into two major groups based on their primary structure. One of histone subtypes, homomorphous variants, display minor differences in their amino acid composition, and are temporally regulated during the various stages of differentiation and

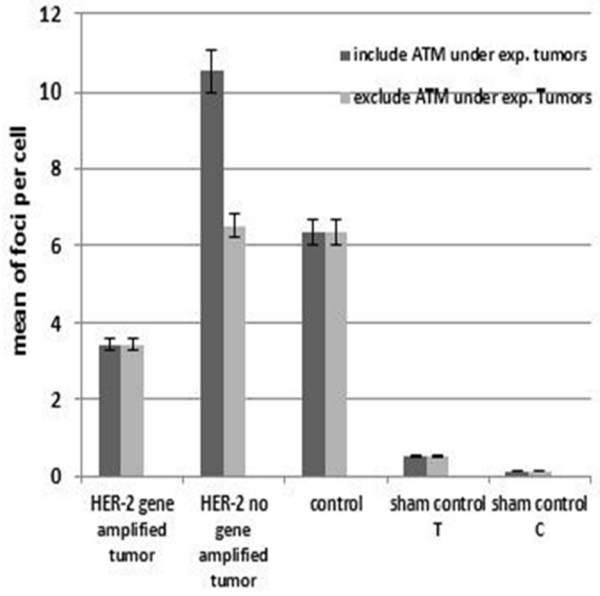


Figure 4. mean of foci manifesting residual DSB frequency in irradiated ductal carcinoma tumors with or without HER-2 gene amplification and control compared non-irradiated sham controls.

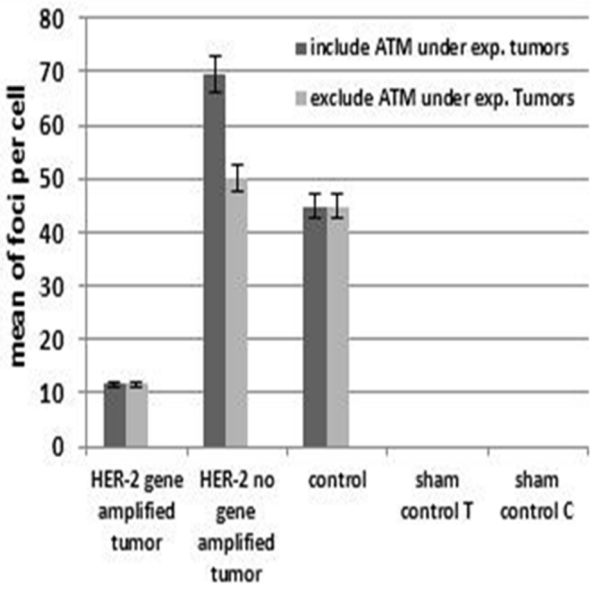


Figure 5. Frequency of cells with ≥5 foci per cell in 4 Gy gamma irradiated ductal carcinoma breast tumor tissues with or without HER-2 gene amplification and control after 24 hours repair time compared to non-irradiated sham control groups.

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cell aging. The other group of histone subtypes, heteromorphous variants which include H2AX, have a different primary structure, in size and/ or amino acid sequence [27].

Each core histone contains tail motifs in both the NH2- and the COOH- terminal regions which are targets for post-translational modification. It has been proposed that the combinatorial nature of histone NH2- and COOH-terminal modifications (which include phosphorylation, acetylation, methylation, ubiquitination and sumoylation) could define a "histone code" which might considerably extend the information potential of the genetic code [28]. According to this hypothesis, this epigenetic marking system represents a fundamental regulatory mechanism that has an impact on most, if not all, chromatin-template processes, with far-reaching consequences for cell fate decisions and normal and pathological development. H2AX is ubiquitously distributed and expressed throughout the genome. H2AX contains a COOH-terminal region longer than those seen in the bulk H2A species. In the past several years, the phosphorylated form of histone H2AX, i.e. γH2AX, which is phosphorylated at serine 139 within the conserved COOH-terminal region, has attracted considerable attention.

An early event in the evolutionarily conserved cellular DNA damage response (DDR) is phosphorylation of H2A histones in chromatin around DSBs [13]. This covalent modification creates binding sites for DDR factors that assemble into complexes around DNA breakage sites and catalyze other histone modifications, which anchor additional DDR proteins near DSBs. Deficiencies in these mechanisms cause DSB repair defects leading to genomic instability that can interfere with normal cellular physiology and cause cell death or malignant transformation. For example, reduced expression of histone H2AX leads to chromosome breaks in nonmalignant cells and immunodeficiency and lymphomas with clonal translocations in mice [29,30]. Despite their importance to normal cellular physiology and relevance to immunology and cancer, the mechanisms through which post-translational histone modifications coordinate DSB repair with cellular proliferation and survival remain enigmatic.

The amount of γH2AX per DSB found in cells corresponds to the phosphorylation of H2AX molecules covering a very large region, estimated to contain about 2 Mb of chromatin, or thousands of nucleosomes [19]. Subsequently, the development of a phospho-specific anti-γH2AX antibody confirmed that H2AX is densely phosphorylated in the chromatin surrounding DSBs, in what is now known as nuclear foci [21]. Initial studies had observed a close correlation between the number of γH2AX foci and the number of expected DSBs after irradiation [21]. Among the variety of possible DNA-damage events which can occur in the cell, DSBs are the most deleterious. It has been established that one DSB remaining unrepaired in a cell can potentially result in cell death.

Previously, DSBs in cellular DNA have been quantitated by pulse-field gel electrophoresis (PFGE) assays, neutral single cell gel electrophoresis (the comet assay), and the DNA elution assay. Since these methods all indicated that the efficiency of DSB detection was very low, the lower limit of DSB detection had been about 100 DSBs per cell nucleus. In addition, these methods were unable to clarify the localization of DSBs within the nucleus [31]. The γH2AX assay, an immunocytochemical assay capable of specifically recognizing γH2AX has become the gold standard for the detection of DSBs [17]. This assay is currently accepted as being an extremely sensitive and specific indicator for the existence of only one DSB: specifically, one γH2AX focus correlates to one DSB [11,21].

γH2AX foci were detected, not only after exposure to IR, but also after exposure to classical DSB-inducing agents such as bleomycin (BLM) [32], tirapazamine [32], etoposide (ETP) [32,33] and doxorubicin (DOX) [34] It was reported that low pH also induces the formation of γH2AX foci [34,35]. It is possible that low pH conditions induce DSBs by preventing topoisomerase II-mediated DNA relegation [35]. However, this mechanism may involve other associated endogenous stresses, because an acidic pH

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induces a high frequency of apoptosis [36]. Other endogenous stresses such as meiotic recombination [37], V(D)J recombination [38], heavy chain class switching [39], apoptotic DNA fragmentation [9], senescence [40] and dysfunctional telomeres [41] may generate DSBs. In addition, the background expression of γH2AX in S/G2-phase cells could be a response to DSBs or stalled replication forks at damage sites introduced during normal DNA replication [42,43]. It is of interest that hyper osmotic stress and heat stress have also been reported to induce the formation of γH2AX foci [33,36, 41,44]. Although the mechanisms by which these types of stress induce DSBs has not yet been clarified.

It has been thought that the inhibition of DNA repair under hyperosmotic conditions can lead to an increase in the number of existing DSBs, because transient DNA strand breaks are continuously created during transcription and replication [44]. However, this finding may also depend on the presence of other types of endogenous cellular stress, because hyperosmotic stress induces apoptosis [36]. Although previous reports showed the presence of heat-induced chromosomal damage (45) and DSB formation [45] occurred only during S-phase, other recent work has reported the detection of heat-induced γH2AX foci, not only in S-phase but also in G_1 and G_2-phase [42]. It was reported that heat stress induces ATM activation, and ATM is known to be activated by the presence of DSB lesions [47], and also that heat-induced H2AX phosphorylation is mediated by ATM and DNA-PK [33]. It has also been confirmed that heat induces DSBs through a different pathway through the production of reactive oxygen species (ROS) [48], and leads to an increase in the number of existing DSBs.

Although hypoxia did not induce apoptosis or any DNA damage detectable with the comet assay, phosphorylation of H2AX occurs with much slower kinetics in response to hypoxic stress [34,36]. It is still necessary to clarify if hypoxia-induced γH2AX depends on the presence of well-known factors such as DSBs or replication arrest, or on the presence of still unknown factors. The identification of additional hypoxia specific targets will permit a more detailed understanding of the γH2AX response.

After exposure to ionizing radiation, histone H2AX molecules in megabase chromatin regions adjacent to break sites are phosphorylated within minutes on serine-139 residues. This phosphorylated form of H2AX, termed γ H2AX, can be visualized by immunofluorescence analysis and forms discrete nuclear foci, which reflect sites of DSBs. γH2AX-foci analysis is a highly sensitive technique to detect DSBs, and the kinetics of γH2AX-foci loss strongly correlate with the time course of DSB repair [11,49]. The phosphorylated form of the histone H2AX, γ -H2AX, occurs at the site of DNA-DSBs. When cells are exposed to high–dose-rate γ-radiation, the number of γ-H2AX foci reaches a peak at around 30 min and then diminishes after the repair of DNA-DSBs [32,43]. The peak number of γ-H2AX foci represents the maximal number of DNA-DSBs induced by radiation, whereas the residual number several hours after irradiation represents the nonrepairable DNA-DSBs and predicts subsequent cell death [32]. It is likely that the kinetics of γ -H2AX foci formation after exposure to internalizing radioisotopes differ markedly from the kinetics of foci formation after high–dose-rate γ-radiation. The number of foci after prolonged exposure to an internalized radioisotope would represent a combination of residual unrepaired DNA-DSBs as well as new DNA-DSBs that are formed as a result of continuing decay of the radionuclide.

As mentioned before, the recruitment of DNA damage signaling and repair proteins to sites of genomic damage constitutes a primary event triggered by DNA damage. Many components of the DNA damage response, including ATM, BRCA1, 53BP1, MDC1, RAD51, and the MRE11/ RAD50/NBS1 complex, form IR-induced foci that co-localize with γH2AX foci. These nuclear micro -domains are thought to contain hundreds to thousands of molecules that accumulate in the vicinity of a DSB [17]. The localization of γH2AX foci is in agreement with its putative role in DSB repair and genomic instability. Therefore, an immunocytochemical assay recognizing the presence of γH2AX could theoretically be used as a sensitive test for the detection of potential carcinogens [50].

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Our results showed that 4 Gy gamma irradiation of breast tissues samples dramatically increased the frequency of residual DSBs even after 24 hours post irradiation repairing time compared to non-irradiated sham control. The frequency of DSBs in ductal carcinoma tumors with ATM under expression was significantly higher than normal ATM expressed ones. This stated that ATM expression to repair of DSBs would be mandatory and it's under expression would lead to more sensitivity of tissues to irradiation. Also In these groups the frequencies of DSB in relevant normal adjacent tissues were higher than control. It means that the expression of ATM in normal tumor adjacent tissues of these patients under express as well as tumor ones that make them more sensitive to ionizing radiations.

ATM has role in repair of double stranded breaks of DNA in two ways: one collaborates with DNA-Pk and the other had interaction with Rad 51.

It has been reported that γH2AX induction following exposure to IR is mediated by ATM and DNA-PK [51]. The phosphorylation of H2AX by ATM occurs at sites of DSBs in the cell nucleus whereas ATM autophosphorylation is thought to take place throughout the nucleoplasm. When ATM interacts with Nbs1 after exposure to IR, ATM transfers from the cytoplasm into the nucleus and contributes to the phosphorylation of H2AX. In recent work, the Nbs1 C-terminal ATM interaction motif was found to be required for the effective phosphorylation of ATM target at DSB sites in DNA. When ATM molecules and/or the C-terminal region of Nbs1 are absent, however, DNA-PK can contribute to the phosphorylation of H2AX without any interaction between ATM and Nbs1. Thus, the independence of H2AX phosphorylation on Nbs1 status reflects an increased contribution by DNA-PK complementation in cells dysfunctional for ATM such as AT cells [52].

In this research the relationship between HER-2 gene amplification and residual induced DSBs in ductal carcinoma breast tissues were also studied. Our results showed that the frequency of residual DSBs after 24 hours post 4 Gy gamma rays was significantly lower compared to normal amplified ones. This stated that more DSB repair was done in HER-2 gene amplified tumors compared to normal amplified ones. In the other statement our results indicated that gamma irradiation sensitivity was lower in HER-2 gene amplified tumors compared to normal amplified ones. This may be due to EGFR downstream signaling pathway that with the use of cell survival mechanisms ends to resistance against radiation effects by repairing of induced DSBs using NHEJ pathway and by activation of PI3K/ACT due to irradiation and finally leads to DSB repair [53].

The different frequency of DSBs observed in the tissues with variety expression and amplification of the genes may indicate the different individual radiotherapy responses and γ H2AX assay as a tool for analyzing DSB works well. But using this technique in vitro such as what we have done in this research to predict radiotherapy responses needs more researches.

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REFERENCES

1. Fernet M, Hall J (2004) Genetic biomarkers of therapeutic radiation sensitivity. *DNA Repair, **3:** 1237 - 43.*
2.Rieger KE, Hong WJ, Tusher VG, Tang J, Tibshirani R, Chu G (2004)Toxicity from radiation therapy associated with abnormal transcriptional responses to DNA damage. *Proc Natl Acad Sci US A,**10:** 6635-40.*
3.Svensson JP, Stalpers LJ, Esveldt-van Lange RE, Franken NA, Haveman J, Klein B, Turesson I, Vrieling H, Giphart-Gassler M (2006) Analysis of gene expression using gene sets discriminates cancer patients with and without late radiation toxicity. *PLoSMed, **3:** e422.*
4. Jackson SP (2002) Sensing and repairing DNA double-strand

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breaks. *Carcinogenesis,* ***23:*** *687–96.*
5. Bentzen SM (2006) Preventing and reducing late side effects of radiation therapy: Radiobiology meets molecular pathology. *Nat Rev Cancer.* ***6:*** *702-13.*
6. Begg AC (2006) Can the severity of normal tissue damage after radiation therapy be predicted? *PLoSMed.* ***3:*** *e440.*
7. Baumann M, Ho« lscherT, Begg AC (2003) Towards genetic prediction of radiation responses: ESTRO GENEPI project. *Radiother Oncol.* ***69:****127-35.*
8. Hall EJ, Giaccia AJ (2006): Radiobiology for the radiologist. 6th edition. Philadelphia: Lippincott Williams & Wilkins.
9. Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM, (2000) Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *J Biol Chem* ***275:*** *9390-9395.*
10. Nazarov IB, Smirnova AN, Krutilina RI, Svetlova MP, Solovjeva LV, Nikiforov AA, Oei SL, Zalenskaya IA, Yau PM, Bradbury EM, Tomilin NV(2003) Dephosphorylation of histone gamma-H2AX during repair of DNA double-strand breaks in mammalian cells and its inhibition by calyculin A. *Radiation Research,* ***160:*** *309-17.*
11.Rothkamm K, Kruger I, Thompson L, Lobrich M (2003) Pathways of DNA double-strand break repair during the mammalian cell cycle. *Molecular Cellular Biology,* ***23:*** *5706 -15.*
12. Rothkamm K, Lobrich M (2003) Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci USA,* ***100:*** *5057-5062.*
13. Kinner A, Wu W, Staudt C, Iliakis G (20080 Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res,* ***36:*** *5678-94.*
14. Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, Naka K, Xia Z, Camerini- Otero RD, Motoyama N, Carpenter PB, Bonner WM, Chen J, Nussenzweig A (2002) DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol,* ***4:*** *993-7.*
15. Lowndes NF and Toh GW (2005) DNA Repair: The importance of phosphorylating histone H2AX. *Curr Biol,* ***15:*** *99-102.*
16. van Attikum H, Gasser SM (2005) The histone code at DNA breaks: a guide to repair? *Nat Rev Mol Cell Biol,* ***6:*** *757-65.*
17. Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A (2004) H2AX: the histone guardian of the genome. *DNA Repair,* ***3****: 959-967.*
18. Bassing CH, Suh H, Ferguson DO, Chua KF, Manis J, Eckersdorff M, Gleason M, Bronson R, Lee C, Alt FW (2003) Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. *Cell.* ***114:*** *359–70.*
19. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem,* ***273:*** *5858-5868*.
20. Sedelnikova O, Rogakou E, Panyutin I, Bonner W (2002) Quantitative detection of (125)IdU-induced DNA double-strand breaks with gamma-H2AX antibody. *Radiation Research,* ***158:*** *486-92.*
21. Rogakou EP, Boon C, Redon C, Bonner WM (1999) Megabase chromatin domains involved in DNA double-strand breaks *in vivo*. *J Cell Biol,* ***146:*** *905-916.*
22. Costes SV, Boissiere A, Ravani S, Romano R, Parvin B, Barcellos-Hoff MH (2006) Imaging features that discriminate between foci induced by high- and low-LET radiation in human fibroblasts. *Radiat Res.****165:*** *505-15.*
23. Salimi M, Mozdarani H, Majidzadeh K (2012) Expression pattern of ATM and cyclin D1 in ductal carcinoma, normal adjacent and normal breast tissues of Iranian breast cancer patients. *Med Oncol.* ***29:*** *1502-9.*
24. Salimi M, Mozdarani H, Majidzadeh-A K (2012) Efficacy of primed in situ labelling in determination of HER-2 gene amplification and CEN-17 status in breast cancer tissue. *Asian Pac J Cancer Prev.* ***13:*** *329-37.*
25. Luger K (2003) Structure and dynamic behavior of nucleosomes. *Curr Opin Genet Dev* ***13:*** *127-135.*
26. Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K, Bonner W (2002) Histone H2A variants H2AX and H2AZ. *Curr Opin Genet Dev,* ***12:*** *162-169.*
27. Lewis JD, DW Abbott, J Ausio (2003) A haploid affair: core histone transitions during spermatogenesis. *Biochem Cell Biol,* ***81:*** *131-140.*
28. Jenuwein T, Allis CD (2001) Translating the histone code. *Science,* ***293:*** *1074-1080.*
29. Celeste A, Difilippantonio S, Difilippantonio MJ, Fernandez-Capetillo O, Pilch DR, Sedelnikova OA, Eckhaus M, Ried T, Bonner WM, Nussenzweig A (2003) H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell.* ***114:*** *371–83.*
30. Bassing CH and Alt FW (2004) H2AX May Function as an Anchor to Hold Broken Chromosomal DNA Ends in Close Proximity. *Cell Cycle,* ***3:*** *149-53.*
31. Takahashi A and Ohnishi T (2005) Dose gammaH2AX foci formation depends on the presence of DNA double strand breaks? *Cancer Lett,* ***229:*** *171-9.*
32. Banath JP, Macphail SH, Olive PL (2004) Radiation sensitivity, H2AX phosphorylation, and kinetics of repair of DNA strand breaks in irradiated cervical cancer cell lines. *Cancer Res,* ***64:*** *7144–7149.*
33. Kaneko H, Igarashi K, Kataoka K, Miura M (2005) Heat shock induces phosphorylation of histone H2AX in mammalian cells. *Biochem Biophys Res Commun,* ***328:*** *1101-1106.*
34. Hammond EM, Dorie MJ, Giaccia AJ (2003) ATR/ATM targets are phosphorylated by ATR in response to hypoxia and ATM in response to reoxygenation. *J Biol Chem.* ***278:*** *12207-12213.*
35. Xiao H, Li TK, Yang JM, Liu LF (2003) Acidic pH induces topoisomerase II-mediated DNA damage. *Proc Natl Acad Sci USA,* ***100:*** *5205-5210.*
36. Hammond EM, Green SL, Giaccia AJ (2003) Comparison of hypoxia-induced replication arrest with hydroxyurea and aphidicolin-induced arrest. *Mutat Res,* ***532****: 205-213.*
37. Mahadevaiah SK, Turner JM, Baudat F, Rogakou EP, de Boer P, Blanco-Rodriguez JJasin, M, Keeney S, Bonner WM, Burgoyne PS (2001) Recombinational DNA double-strand

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breaks in mice precede synapsis. *Nat Genet,* ***27:*** *271-276.*
38. Klein S, Zenvirth D, Dror V, Barton AB, Kaback DB, Simchen G (1996) Patterns of meiotic double-strand breakage on native and artificial yeast chromosomes. *Chromosoma,* ***105:*** *276-284.*
39. Petersen S, Casellas R, Reina-San-Martin B, Chen HT, Difilippantonio MJ, Wilson PC, Hanitsch L, Celeste A, Muramatsu M, Pilch DR, Redon C, Ried T, Bonner WM, Honjo T, Nussenzweig MC, Nussenzweig A (2001) AID is required to initiate Nbs1/γ-H2AX focus formation and mutations at sites of class switching. *Nature,* ***414:*** *660-665.*
40. Sedelnikova OA, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC (2004) Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. *Nat Cell Biol,* ***6:*** *168-170.*
41. Hao LY, Strong MA, Greider CW (2004) Phosphorylation of H2AX at short telomeres in T cells and fibroblasts. J Biol Chem, **279**: 45148-45154.
42. Takahashi A., Matsumoto H., Nagayama K., Kitano M., Hirose S., Tanaka H., Mori E., Yamakawa N., Yasumoto J., Yuki K., Ohnishi K., Ohnishi T. (2004) Evidence for the involvement of double-strand breaks in heat-induced cell killing. *Cancer Res,* ***64:*** *8839-8845.*
43. Mac Phail SH, Banath JP, Yu Y, Chu E, Olive PL (2003) Cell cycle-dependent expression of phosphorylated histone H2AX: reduced expression in unirradiated but not X-irradiated G1-phase cells. *Radiat. Res,* ***159:*** *759-767.*
44. Dmitrieva NI, Bulavin DV, Burg MB (2003) High NaCl causes Mre11 to leave the nucleus, disrupting DNA damage signaling and repair. *Am J Physiol Renal Physiol,* ***285:*** *F266-F274.*
45. Wong RS and Dewey WC (1993) Molecular mechanisms for the induction of chromosomal aberrations in CHO cells heated in S phase. *Environ Mol Mutagen,* ***22:*** *257-263.*
46. Wong RS, Dynlacht JR, Cedervall B, Dewey WC (1995) Analysis by pulsed-field gel electrophoresis of DNA double-strand breaks induced by heat and/or X-irradiation in bulk and replicating DNA of CHO cells. *Int J Radiat. Biol,* ***68:*** *141-152.*
47. Miyakoda M, Suzuki K, Kodama S, Watanabe M (2002) Activation of ATM and phosphorylation of p53 by heat shock. *Oncogene,* ***21:*** *1090-1096.*
48. Bruskov VI, Malakhova LV, Masalimov ZK, Chernikov AV (2002) Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA. *Nucleic Acids Res,* ***30:*** *1354-1363.*
49. Mahrhofer H, Bürger S, Oppitz U, Flentje M, Djuzenova CS (2006) Radiation induced DNA damage and damage repair in human tumor and fibroblast cell lines assessed by H2AX phosphorylation. *Int J Radiat Oncol Biol Phys,* ***64:****573-80.*
50. Albino AP, Huang X, Jorgensen E, Yang J, Gietl D, Traganos F, Darzynkiewicz Z (2004) Induction of H2AX phosphorylation in pulmonary cells by tobacco smoke: a new assay for carcinogens. *Cell Cycle* ***3****: 1062-1068.*
51. Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA (2004) ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res,* ***64:*** *2390-2396*.
52. Falck J, Coates J, Jackson SP (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature,* ***434****: 605-611.*
53. Toulany M and Rodemann HP (2010) Membrane receptor signaling and control of DNA repair after exposure to ionizing radiation. *Nuklearmedizin,* ***49 Suppl 1:*** *S26-30.*

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