MMSL Military Medical Science Letters

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2011, vol. 80(4), p. 169-177
ISSN 0372-7025
DOI: 10.31482/mmsl.2011.023

REVIEW ARTICLE

ROLE OF γ-H2AX IN DNA-DAMAGE RESPONSE AND ITS POSSIBLE CLINICAL APPLICATIONS

Eva Novotna[1]✉, Ales Tichy[1], Karolina Foltanova[2], Jirina Vavrova[1]

[1] Department of Radiobiology, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic
[2] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic

Received 17th October 2011.
Revised 29th November 2011.
Published 9th December 2011.

Summary

The integrity of the human genome is constantly threatened by exogenous or endogenous genotoxic agents that cause DNA damage. The ionizing radiation (IR)-induced DNA double-strand breaks (DSBs) are considered as the most deleterious forms of DNA damage which could lead to genomic instability and to cancer development, if left unrepaired. The DNA damage response (DDR) is comprised of a network of proteins that cooperate to regulate cell cycle progression and repair of DNA lesions. Our understanding of molecular basis of repair processes and of functions of repair proteins, as well as understanding of chromatin modifications may provide new possibilities in improvement of cancer management. Phosphorylation of histone variant H2AX at serine 139 (γ-H2AX) and formation of γ-H2AX repair foci seems to be the most sensitive DNA damage marker in the chromatin flanking the free DNA double-stranded ends in DSBs. Monitoring of γ-H2AX levels can serve for early indication of cancer development, as biomarker of cancer therapy efficiency or as a biodosimetric marker of radiation exposure.

Key words: DNA damage response; ionizing radiation; double-strand breaks; γ-H2AX, cancer biomarker; biodosimetry

ABBREVIATIONS

53BP1, p53 binding protein 1;
A-T, ataxia telangiectasia;
ATM, ataxia telangiectasia mutated protein kinase;
ATR, ATM and Rad3-related protein kinase;
BRCA1, breast cancer 1;
tBRCT, C-terminal tandem BRCA1domain;
CDKN1A, cyclin dependent kinase inhibitor 1 A;
Chk 1/2, checkpoint kinase 1/2;
CT, computed tomography;
DDR, DNA damage response;
DNA-PK, DNA-dependent protein kinase;
DSB, double strand break;
EYA1/3, eyes absent homolog 1/3 tyrosine phosphatase
HR, homologous recombination;
IR, ionizing radiation; IRIF, ionizing radiation-induced foci;
JNK-1, C-Jun N-terminal protein kinase;
KAP-1, KRAB-ZFP–associated protein 1;
MRN, Mre11/Rad50/NBS1complex;
MDC1, mediator of DNA damage checkpoint 1;
NHEJ, non-homologous end joining;
PIKKs, Phosphatidylinositol-3-kinase-related kinases;
RNF8/168, ring finger protein 8/168
ROS, reactive oxygen species;

✉ University of Defence, Faculty of Military Health Sciences, Department of Radiobiology, Třebešská 1575, 500 01 Hradec Králové, Czech Republic
novotnae@pmfhk.cz
☎ +420 973253217

SSB, single strand break;
UBC13, ubiquitin conjugating enzyme 13
XRCC4, X-ray repair complementing defective repair in Chinese hamster cells 4;
WSTF, Williams-Beuren syndrome transcription factor

INTRODUCTION

Human DNA is constantly exposed to a broad variety of mutagenic and genotoxic agents of endogenous or exogenous origin, including ionizing radiation (IR). Most of these IR come from natural resources like radioactive elements in ground, cosmic or sun rays or natural radionuclides in the human body. The growth in usage of radiation for industrial purposes increases the risk of accidental exposure from industrial accidents, such as recent accident in Fukushima. Further to that, the possibilities of its abuse as a weapon of terrorism as well as testing of nuclear weapons in the atmosphere, threaten to cause individual IR exposure.

Medical usage of irradiation (X-ray, CT scanning) contributes only little to an overall exposure, because of relatively low doses of irradiation being used [1]. Furthermore, IR used in radiotherapy has been a powerful tool for cancer treatment. On the other hand, it has been demonstrated that exposure to IR enhances cancer incidence and radiation represents a mild carcinogen [1,2]. Moreover, there is accumulating evidence that doses obtained during CT scanning can contribute to the background level of carcinogenesis [2].

There is no doubt that DNA is the main target of radiation exposure and IR-induced DNA damage can lead to genomic instability and carcinogenesis. Therefore, there is a strong need for reliable biomarkers of such an extremely dangerous DNA damage. Recently, several proteins involved in DNA damage response (DDR) and signalling have been suggested as the potential indicators of IR exposure. Expression of these proteins correlates with dynamics of IR-induced damage repair, which means their increase is dose-dependent and they are supposed to be expressed for several days after IR exposure until efficient damage repair is completed [3]. According to recent studies, proteins γ-H2AX, ATM kinase, p53, 53 binding protein 1 (53BP1) and CDKN1A have been proposed as the most comprehensive indicators of radiation exposure [4-6]. In this review, we are focusing on functional roles of γ-H2AX in DDR and its potential clinical applications.

Molecular mechanisms of DNA damage response

DNA strand breaks

There are many types of IR-induced DNA damage, such as base modifications, DNA crosslinks or strand breaks. Each type of DNA damage posses specific set of cellular responses to deal with the nature of the damage. In the context of radiobiology, exposure to IR leads to generation of the DNA single-strand (SSBs) and double-strand breaks (DSBs), where DSBs are considered as the most critical forms of all DNA lesions. Although base damages and SSBs are the most numerous DNA lesions, there are overlapping repair pathways involved in their eradication. These lesions are biologically significant during DNA replication, thus they could potentially contribute to promotion of increased mutational events.

On the other hand, DSBs are less numerous, but it has been shown that even one DSB can lead not only to mutations, genomic instability and terminally to the cell death, but also to malignant transformation of the cell in the case of inefficient DSBs repair [7,8].

DNA DSBs can arise not only after exposure to IR. They are physiologically induced during selective cellular processes such as meiotic recombination or immunoglobulin gene rearrangement (V (D) J recombination). They could also be generated by exposure to endogenous agents, such as reactive oxygen species (ROS) produced by cellular respiration, or exogenous genotoxic agents, such as cytostatics used in oncology [9].

Double strand breaks repair pathways

Several DNA repair pathways have been developed during cellular evolution. The major DSBs repair pathway in mammalian cells is non-homologous end joining (NHEJ), which predominates in non-replicating cells in G1/G0 phase of cell cycle, although it is also involved in repairs in other phases of the cell cycle [10-12]. This repair pathway consists of break recognition, DNA processing and finally of ligation of processed DNA ends [13]. NHEJ is activated by the binding of Ku70/80 heterodimer to double strand (ds) DNA ends. The binding of Ku heterodimer recruits catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) to generate active protein kinase DNA-PK. DNA-PK is probably the key regulator of NHEJ, because its autophosphorylation leads to coordination of end-processing and end-joining, and regulation of the NHEJ processes [13,14].

Other key proteins involved in the end-processing are endonuclease Artemis and DNA ligase IV with its cofactors XRCC4 and XLF-Cernunos, which are responsible for the final ds DNA end-joining.

The second pathway involved in DSBs repair is homologous recombination (HR) [12]. This pathway has a major role at the replication fork, which promotes restart after replication fork stalls or collapse [15], and is also important for repair of regions of single stranded (ss) DNA arising from replication fork collapsing. HR was commonly believed as a major DSBs repair pathway in G2 phase, but a recent study has shown that HR only functions in the repair of 15 % of radiation induced DSBs while NHEJ predominates in DSBs repair in G2 phase [16].

Phosphatidylinositol-3-kinase-related kinases (PIKKs) as the main factors of DDR

Within a few minutes after IR exposure and DNA damage recognition, three protein kinases, ataxia telangiectasia mutated-protein kinase (ATM), ATM and Rad3-related protein kinase (ATR), and DNA-PK (described above) are activated to orchestrate the repair processes. Whilst DNA-PK is crucial in NHEJ, ATM is conducted with HR, and it has emerged as a master kinase for DNA damage signal transduction and repair [17].

ATM is critical for the initiation of DDR and the mutation in ATM gene leads to a rare childhood disorder *Ataxia telangiectasia* (A-T), which is characterised by the profound sensitivity to IR, neurodegeneration and predisposition to cancer [18]. The kinase activity of ATM is extremely sensitive to DNA damage and is activated and autophosphorylated within a few minutes after IR due to interaction with Mre11/Rad50/NBS1 (MRN) complex, which represents the initial DSB sensor [19]. Phosphorylation of ATM at serine 1981 is essential for the activation of downstream effector kinases, such as checkpoint kinases Chk1/2 and p53, proteins responsible for cell cycle arrest. Another function of ATM is modification of nuclear heterochromatin and histones in order to facilitate the access of the repair proteins to the damaged DNA.

Nuclear chromatin presents a complex structure of DNA and proteins localised in cell nucleus. The dynamic organisation of chromatin structure thereby influences, potentially, all functions of the genome. There are two forms of chromatin organisation: transcriptionally active *eu*chromatin is usually localised in the interior of nucleoplasm, while highly condensed and functionally distinct *hetero*chromatin, which comprises 10-25 % of total chromatin (depending on cell type, age, etc.) [20], localises at the periphery of the nucleus. Interestingly, recent study of Goodarzi et al. [21] suggested that heterochromatin could be a barrier to DSB repair, thus ATM overcomes this barrier by phosphorylation of KAP-1, a core factor of heterochromatin formation.

One of the first chromatin modifications induced by ATM is phosphorylation of histone H2AX at serine 139, commonly abbreviated as γ-H2AX [22]. Within a few minutes after IR and DNA damage recognition, γ-H2AX spreads along the chromosome and incorporates with another proteins to form discrete structures called "ionizing radiation induced foci" (IRIF) or γ-H2AX foci , where the repair proceeds [21, 23]. γ-H2AX interacts with an adaptor protein MDC1 (mediator of DNA damage checkpoint protein 1), that senses γ-H2AX and orchestrates assembly of all essential repair proteins (such as MRN, 53BP1 etc.) on the chromatin at the site of DSB [24].

As well as ATM, DNA-PK was shown to be involved in H2AX phosphorylation and MDC1 and 53BP1 activation [25]. However, it cannot substitute for ATM in phosphorylating other substrates such as Chk2 or p53.

Characterisation of γ-H2AX as a key regulator of DDR

Central role of H2AX phosphorylation among chromatin post-translational modifications

Because of its length (~ 2 m), nuclear DNA is wrapped around an octamer of histones and creates the basic structure of chromatin, the nucleosome. Histone H2AX is a variant of the H2A histone family and comprises 10-15 % of total cellular H2A in higher eukaryotes [26]. Each member of H2A family occupies an analogous position in different nucleosomes, which leads to considerable nucleosomal diversity.

The central globular domain of H2AX is flanked by amino- and carboxyl-terminals where the post-translational modifications could be carried out [27]. In contrast to the structurally similar H2A histones, H2AX contains four serine residues from the C-terminus (omega-4) of the protein (SQEY-COOH motif), which become phosphorylated upon induction of DSBs to form γ-H2AX [26,28]. In addition, serine 139 (S139) phosphorylation in H2AX occurs at the carboxyl terminus of the his-

tone, instead of the more frequently modified amino-terminus [29].

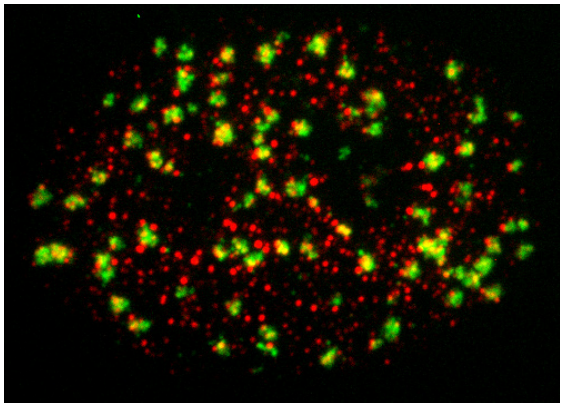
More recently, it has been found that tyrosine residue 142 (Y142) of H2AX is also phosphorylated after IR induced DNA damage via Williams-Beuren syndrome transcription factor (WSTF), and this phosphorylation seems to play the opposite role to γ-H2AX [30, 31]. While γ-H2AX recruits MDC1 and other repair factors, in cases of irreparable damage, Y142 is re-phosphorylated by tyrosine phosphatase EYA1/3, and this re-phosphorylation inhibits MDC1 recruitment to DSB. Simultaneously, pro-apoptotic JNK1 complex is recruited to promote programmed cell death. However, the regulation of this re-phosphorylation remains unclear [32].

Besides the central role of phosphorylation, other post-translational modifications of H2AX, such as acetylation/deacetylation, ubiquitylation or methylation, also participate in DDR [33].

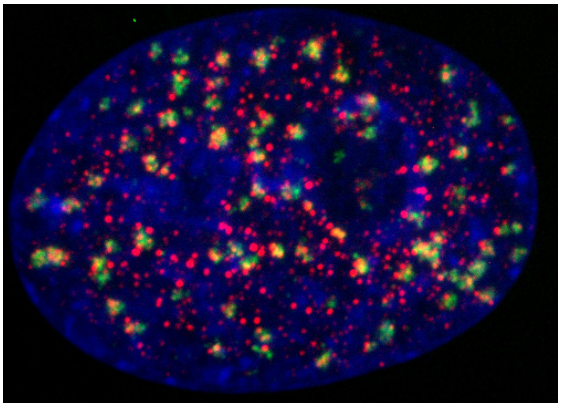
Mediator of DNA damage checkpoint protein 1 (MDC1) and γ-H2AX

Since the recognition of γ-H2AX in controlling DNA damage repair, several DDR proteins were shown to possess specific domains that can interact in phosphorylation-dependent manner. Among the various domains of DDR proteins, the carboxyl-terminal tandem (tBRCT) domain of MDC1 protein was found to be the predominant γ-H2AX binding partner [24, 34]. MDC1 is a specific sensor of γ-H2AX, because its tBRCT domain shows high specificity towards the phosphorylated form of SQEY-COOH motif of H2AX. Due to its strong binding, MDC1 protects γ-H2AX from de-phosphorylation and mediates downstream γ-H2AX signals [35]. MDC1 forms foci that co-localise extensively with γ-H2AX foci within minutes after exposure to ionizing radiation, and this co-localisation can be detected immunohistochemically by specific antibodies (See Fig.1).

Figure 1. γ-H2AX-MDC1 co-localisation (foci) in cell nucleus.
HeLa cancer cells (cervical carcinoma) were irradiated with 8 Gy in the presence of DNA-PK inhibitor.



A. Repair foci were visualised by fluorescent-labelled antibodies against γ-H2AX (FITC-green) and MDC1 (Cy3-red) at site of DSB (merge-yellow).



B. Repair foci and nuclear chromatin. Chromatin was visualised by TOPRO3 (blue).
Magnification 1000x

In addition, MDC1 serves as the master recruitment platform for repair proteins, such as 53BP1, BRCA1 and MRN. Interaction with MRN leads to the recurrent ATM activation at the DSB, which in turn phosphorylates more distal H2AX molecules [36]. MDC1 activates the ubiquitylation of histones (ubiquitin ligase cascade RNF8-RNF168-UBC13), thus activating the promotion of 53BP1 and BRCA1 repair proteins to assemble at IRIF [37]. RNF8 interacts with MDC1 and subsequently with RNF168 and UBC13 to activate the ubiquitylation, which leads to recruitment of BRCA1 to the site of DSB. Ubiquitylation of histones and/or other proteins at site of DNA damage might expose modified histones and facilitate the recruiting of 53BP1, which binds to methylated histones [38, 39].

Another function of MDC1 is in controlling DNA damage-induced cell-cycle arrest checkpoints [34]. It has been found that downregulation of MDC1 causes improper activation of G2/M and

intra-S-phase checkpoints and affects IR-induced apoptosis [40].

The role of MDC1 in Chk2 activation still remains unclear, because Chk2 phosphorylation is ATM-dependent; it does not accumulate at DSB and spreads throughout the nucleoplasm to perform its effector activities. Therefore, it is possible that MDC1 interacts indirectly with both the kinases and transiently bridges the release of phosphorylated Chk2 [41].

Other functions of γ-H2AX

Besides its essential role in recruitment and accumulation of DNA repair proteins, γ-H2AX was shown to interact with other DDR proteins. Interaction of γ-H2AX with cohesins helps to maintain chromatid cohesion while DNA is being repaired [42]. In addition, γ-H2AX maintains checkpoint responses during DSB repair, and is necessary for activation of checkpoints after the exposure to low-dose IR [43,44]. In the case of irreparable damage, γ-H2AX contributes to cellular apoptosis [45].

Clinical applications of γ-H2AX

γ-H2AX as a biomarker

Since phosphorylation of H2AX is considered as one of the most important steps in initiation and signalling of DSB repair, the decline in the number of γ-H2AX foci is believed to reflect the kinetics of the repair processes [46]. Furthermore, the ratio of γ-H2AX to DNA DSB is close to 1:1 [47], thus it can serve as a sensitive biomarker for IR-induced DNA damage [6,48], and can be used as a therapeutic marker of cancer or a possible target of anticancer therapies [49,50].

Moreover, it could serve as a biodosimetric marker in the prediction of IR-doses delivered to individuals during nuclear or radiological accidents or attacks [4]. The level of γ-H2AX in rat peripheral blood lymphocytes after whole body or thoracic irradiation was found to be dose-dependent, thus it can be a prompt and reliable marker of the received radiation dose [51,52]. In addition, the opportunity arose to evaluate γ-H2AX biodosimetry in a study using non-human primates subjected to total-body irradiation in the non-lethal to lethal dose ranges [53]. Using realistic scenarios for accidental exposures, the authors showed that γ-H2AX analysis in peripheral blood lymphocytes and plucked hair follicles (eyebrows and whiskers) may be useful for estimation of total-body radiation dose at times at least 4-days post-exposure at doses of 3.5 Gy and above, and evaluated plucked hair as a valid sample material in clinical trials investigating genotoxic effects of radiation exposures or radiation treatments.

Persistence of γ-H2AX foci after the first irradiation is often associated with insufficient and/or defective DNA repair, which often occurs in radiosensitive (A-T or immunodeficient) patients or in patients undergoing radiotherapy. Sometimes, individuals may be sensitive to low dose exposure used in diagnostic CT or X-ray scans, thus γ-H2AX foci could serve as a prediction of radiosensitivity and allow for optimising treatment and diagnostics of individual patient [54].

Furthermore, γ-H2AX has been reported as a potential biomarker for cancer diagnostics or cancer recurrence [49]. For clinical tumour detection, tumour-specific and tumour-associated antigens (e.g. prostatic specific antigen, carcinoembryonic antigen etc.) are frequently monitored, but there are some limitations concerning their use in clinical practice [55]. However, none of these markers possesses 100% specificity for each type of tumour, because of cross-reactivity with other types of tumours or with normal tissues. The presence of elevated levels of γ-H2AX was found in biopsies of human colon, ovary, breasts, liver and kidney tumours, suggesting increased level of DNA damage is a general characteristic of cancer development [49, 56-58]. Moreover, increased level of γ-H2AX was found in chronic inflammatory disease ulcerative colitis, which often predates the development of colorectal cancer [59]. Therefore, detection of γ-H2AX in human samples could serve as a biomarker for early cancer screening.

γ-H2AX as an indirect therapeutic target

As one of the essential regulators of DDR, γ-H2AX could be a potential target to enhance the efficiency of clinical radiotherapy by inhibiting DNA repair in tumour cells [60]. Because all cells contain H2AX in their chromatin structures, and H2AX is structurally and sequentially very similar to histones of H2A family, the protein H2AX itself could be a problematic drug target. On the other hand, inhibition of H2AX phosphorylation could be a more practical strategy for clinical purposes.

Inhibition of H2AX phosphorylation through interference with upstream kinases ATM or DNA-PK has been shown to sensitise tumour cells to IR [61].

Caffeine and a fungal metabolite wortmannin were the first described and tested ATM inhibitors with radiosensitizing effects [62, 63]. More recently, small molecular inhibitors of ATM and DNA-PK were evaluated as more selective and specific agents which significantly sensitised the cytotoxic effects of irradiation or DSBs-inducing chemotherapeutics in pre-clinical evaluation [64,65].

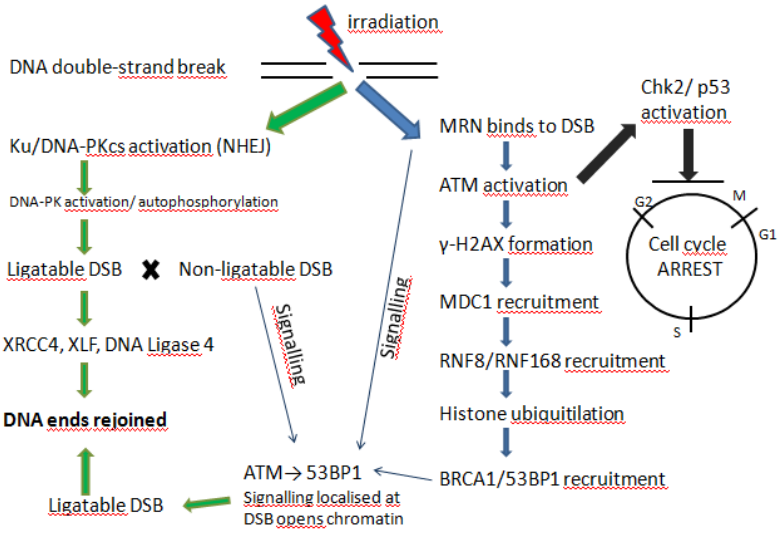


Figure 2. Schematic description of DNA double-strand break response.
Left: DNA-PK signalling. NHEJ factors Ku/DNA-PKcs are activated after recognition of DSB and start DNA end processing. Autophosphorylation of activated DNA-PK leads to activation of XRCC4,XLF, Ligase 4-joining complex and rejoins ligatable DNA ends.
Right: ATM signalling response involves DSB recognition by the MRN complex and the initial activation of ATM. ATM activates both Chk2 and p53 to trigger cell cycle arrest. ATM (and additionally DNA-PK) phosphorylates H2AX to form γ-H2AX. The presence of γ-H2AX recruits MDC1 which, in turn, enables RNF8/168 to activate ubiquitylation of histones and enables the concentration of BRCA1 and 53BP1 at DSB.
Centre: The localised action of ATM and 53BP1 promotes enabling DSB repair of ligatable and (processed) non-ligatable DNA ends.

CONCLUSIONS

γ-H2AX is a sensitive indicator of DNA DSBs and one of the most important regulators of DNA damage repair. Therefore, it could be a sensitive indicator of genotoxic stress. Our understanding of molecular mechanisms and signalling pathways involved in DDR, as well as understanding of chromatin modification and functional roles of γ-H2AX may be useful in clinical applications, especially in cancer therapy or biological dosimetry.

Recently, new monitoring techniques have been developed to facilitate γ-H2AX detection in clinical practice. Although targeting of γ-H2AX and its effectors kinases were described as powerful therapeutic targets, further investigation in the field of cellular responses to DNA damage is needed to identify new target proteins for anticancer therapy.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Specific Research Grant) and by Ministry of Defence of the Czech Republic (Project No. MO0FVZ0000501).

REFERENCES

1. Shurylak I.; Sachs R.K.; Hlatky L.; Little M.P.; Hahnfeldt P.; Brenner D.J. Radiation-induced leukemia at doses relevant to radiation therapy: modelling mechanism and estimating risks. *J. Natl. Cancer Inst.* **2006**, 98, 1794-1806.
2. Hall E.J.; Brenner D.J. Cancer risks from diagnostic radiology. *Br. J. Radiol.* **2008**, 81, 362-378.

3. Kiang J.G.; Garrison B.R.; Gorbunov N.V. Radiation combined injury: DNA damage, apoptosis and autophagy. *Adapt. Med.* **2010**, 2, 1-10.
4. Marchetti F.; Coleman M.A.; Jones I.M.; Wyrobek A.J. Candidate protein biodosimeters of human exposure to ionizing radiation. *Int. J. Radiat. Biol.* **2006**,82, 605-639.
5. Dickey J.S.; Redon Ch.E.; Nakamura A.J.; Baird B.J.; Sedelnikova O.A.; Bonner W.M. H2AX: functional roles and potential applications. *Chromosoma* **2009**, 118, 683-692.
6. Redon Ch.E.; Dickey J.S.; Bonner W.M.; Sedelnikova O.A. γ-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin. *Adv. Space Res.* **2009**, 43, 1171-1178.
7. Bennett C.B.; Lewis A.L.; Baldwin K.K.; Resnick M.A. Lethality induced by a 1017 single site-specific double-strand break in a dispensable yeast plasmid, 1018. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, 90, 5613–5617.
8. Ward J.F. DNA damage and repair. In: W.A.G. and M.N. Varna (Ed.). Physical and Chemical Mechanisms in Molecular Radiation Biology. *Plenum Press, New York* **1991**, 403-421.
9. Collis J.S.; DeWeese T.L.; Jeggo A.P.; Parker A.R. The live and death of DNA-PK. *Oncogene* **2005**, 24, 949-961.
10. Wyman C.; Kanaar R. DNA double-strand break repair: all´s well that ends well. *Annu. Rev. Genet*. **2006**, 40, 363-383.
11. Valerie K.; Povirk L.F. Regulation and mechanisms of mammalian double-strand break repair. *Oncogene* **2003**, 22, 5792-5812.
12. Takata M.; Sasaki M.S.; Sonoda E.; Morrison C. et al. Homologous recombination and nonhomologous end joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J.* **1998**, 17, 5497-5508.
13. Dobbs T.A; Tainer J.A.; Lees-Miller S. A structural model for regulation of NHEJ by DNA-PKcs autophosphorylation. *DNA Repair* **2010**, 9, 1307-1314.
14. Meek K.; Douglas P.; Cui X.; Ding Q.; Lee-Miller S. Trans-autophosphorylation at DNA-dependent protein kinase´s two major autophosphorylation site clusters facilitates end processing but not end joining. *Mol. Cell. Biol.* **2007**, 27, 3881-3890.
15. Petermann E.; Helleday T. Pathways of mammalian replication fork restart. *Nat. Rev. Mol.Cell Biol.* **2010**, 11, 683-687.
16. Beucher A.; Birraux L.; Tchouandong O,; Barton O.et al. ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *EMBO J.* **2009**, 28, 3413-3427.
17. Shrivastav M.; DeHaro L.P.;nickoloff J.A.Regulation of DNA double-strand break repair pathway choice. *Cell Res*. **2008**,18,137-147.
18. McKinnon P.J. ATM and ataxia telangiectasia. *EMBO Reports*, **2004**, 5,772-776.
19. Lamarche B.J.; Orazio N.I.; Weitzman M.D. The MRN complex in double-strand break repair and telomere maintenance. *FEBS Lett.* **2010**, 584, 3682-3695.
20. Milkos G.L.; John B. Heterochromatin and satellite DNA in man: properties and prospects. *Am. J. Hum. Genet.* **1979**, 31, 264-280.
21. Goodarzi A.A.; Jeggo P.; Lobrich M. The influence of heterochromatin on DNA double strand break repair: Getting strong, silent type to relax. *DNA Repair* **2010**, 9, 1273-1282.
22. Rogakou E.P.; Pilch D.R.; Orr A.H.; Ivanova V. S.; Bonner W.M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* **1998**, 273, 5858-5868.
23. Sedelnikova O.A.; Pilch D.R.; Redon C.; Bonner W.M. Histone H2AX in DNA damage repair. *Cancer Biol. Ther.* **2003**, 2, 233-235.
24. Stucki M.; Jackson S.P. γ-H2AX and MDC1: Anchoring the DNA-damage-response, machinery to broken chromosomes. *DNA Repair* **2006**, 5, 534-543.
25. Stiff T.; O´Driscoll M.; Rief N.; Iwabuchi K.; Lobrich M.; Jeggo P. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res.* **2004**, 64, 2390-2396.
26. Fernandez-Capetillo O.; Lee A.; Nussenzweig M.; Nussenzweig A. H2AX: the histone guardian of the genome. *DNA Repair* **2004**, 3, 959–967.
27. Cheung P.; Allis C.D.; Sassone-Corsi P. Signalling to chromatin through histone modifications. *Cell* **2000**, 103, 263-271.
28. Redon C.; Pilch D.; Rogakou E.; Sedelnikova O.A.; Newrock K.; Bonner W. Histone H2A variants H2AX and H2AZ. *Curr. Opin. Genet.* **2002**, 12, 162-169.
29. Kinner A.; Wu W.; Staudt Ch.; Iliakis G. γ-H2AX in recognition and signalling of DNAdouble-strand breaks in the context of chromatin. *Nucl. Acid Res.* **2008**, 36, 5678-5694.
30. Cook P.J.; Ju B.G.; Telese F.; Wang X.; Glass C.K.; Rosenfeld M.G. Tyrosine dephosphoryla-

tion of H2AX modulates apoptosis and survival decisions. *Nature* **2009**, 458, 591–596.
31. Xiao A.; Li H.; Shechter D.; Ahn S.H.; Fabrizio L.A.; Erdjument-Bromage H.; Ishibe-Murakami S.; et al. WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. *Nature* **2009**, 457, 57–62.
32. Lukas J.; Bartek J. DNA repair: New tales of an old tail. *Nature* ,**2009**, 458, 581-583.
33. Luijsterburg M.S.; van Attikum H. Chromatin and the DNA damage response: The cancer connection. *Molecular Oncology* **2011**, 5, 349-367.
34. Steward G.S.; Wang B.; Bignell C.R.; Taylor A.M.R.; Elledge S.J. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* **2003**, 421, 961-966.
35. Stucky M.; Clapperton J.; Mohannad D.; Yaffe M.; Smerdon S.; Jackson S. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* **2005**, 123, 1213-1226.
36. Mochan T.A.; Venere M.; DiTullio R.A.; Halazonetis T.D. 53BP1 and NFBD1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. *Cancer Res.* **2003**, 63, 8586-8591.
37. Bekker-Jensen S.; Mailand N. Assembly and function of DNA double-strand break repair foci in mammalian cells. *DNA Repair*, **2010**, 1219-1228.
38. Mainland N.; Bekker-Jensen S.; Faustrup H.; Melander F.; Bartek J.; Lukas C.;et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* **2007**, 131, 887-900.
39. Doil C.; Mainland N.; Bekker-Jensen S.; Menard P.; Larsen D.H.; Pepperkok R.; et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* **2009**, 136, 435-446.
40. Lou Z.; Minter-Dykhouse K.; Wu X.; Chen J. MDC1 is coupled to activated CHK2 in mammalian DNA damage response pathways. *Nature* **2003**, 421, 957-961.
41. Lou Z.; Minter-Dykhouse K.; Franco S.; Gostissa M.; Rivera M.; Celeste A.; et al. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. *Mol. Cell* **2006**, 21, 187-200.
42. Unal E.; Arbel-Eden A,; Satter U.; Shroff R.; Lichten M.; Haber J.E.; Kohsland D. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol. Cell* **2004**, 16, 991-1002.
43. Downey M.; Durocher D. GammaH2AX as a checkpoint maintenance signal. *Cell Cycle* **2006**, 5, 1376-1381.
44. Fernandez-Capetillo O.;Chec H.T.; Celeste A. et al. DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat. Cell. Biol*. **2002**, 4, 993-997.
45. Mukherjee B.; Kessinger C.; Kobayashi J.; Chen B.P.; Chen D.J.; Chatterjee A.; Burma S. DNA-PK phosphorylates histone H2AX during apoptotic DNA fragmentation in mammalian cells. *DNA Repair* **2006**, 5, 575-590.
46. Rothkamm K.; Lobrich M. Evidence of lack of DNA double-strand break repair in human cells exposed to very low X-ray doses. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 5057-5062.
47. Sedelnikova O.A.; Rogakou E.P.; Panyutin I.G.; Bonner W.M. Quantitative detection of (125)IdU-induced DNA double-strand breaks with gamma-H2AX antibody. *Radiat. Res*. **2002**, 158, 486-492.
48. Horn S.; Rothkamm K. Candidate protein biomarkers as rapid indicators of radiation exposure. *Radiation Measurements* **2011**.
49. Sedelnikova O.A.; Bonner W.M. γ-H2AX in cancer cells: a potential biomarker for cancer diagnostics, prediction and recurrence. *Cell Cycle* **2006**, 5, 2909-2913.
50. Halicka H.D.; Ozkaynak M.F.; Levendoglu-Tual O.; et al. DNA damage response as a biomarker in treatment of leukemia. *Cell Cycle* **2009**, 8, 1720-1724.
51. Havelek R.; Řezáčová M.; Šinkorová Z.; Zárybnická L.; Tichý A.; Vávrová J. Phosphorylation of histone H2AX as an indicator of received dose of gamma radiation after whole-body irradiation of rats. *Acta Vet. Brno* **2011**, 80, 113-118.
52. Havelek R.; Řezáčová M.; Šinkorová Z.; Zárybnická L.; Pejchal J..; Vávrová J. Phosphorylation of histone H2AX in peripheral blood mononuclear cells after thoracic irradiation of rats. *J. Appl. Biomed*. **2011**, 9, 209-218.
53. Redon C.E.; Nakamura A.J.; Gouliaeva K.; Rahman A.; Blakely W.F.; Bonner W.M. The Use of Gamma-H2AX as a biodosimeter for Total-Body Radiation Exposure in non-Human Primates. *Plos One*, **2010**, 5, e15544.
54. Goodarzi A.A.; Jeggo P.A. Irradiation induced foci (IRIF) as a biomarker for radiosensitivity. *Mutat. Res.: Fundam. Mol. Mech. Mutagen*. **2011**, doi:10.1016/j.mrfmmm.2011.05.017.
55. Chatterjee S.K.; Zetter B.R. Cancer biomarkers: Knowing the present and predicting the future. *Future Oncology* **2005**, 1, 37-50.

56. Bartkova J.; Horejsi Z.; Koed K.; et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* **2005**, 434, 864-870.
57. Warters R.L.; Adamson P.J.; Pond C.D.; Leachman S.A. Melanoma cells express elevated levels of phosphorylated histone H2AX foci. *J. Invest. Dermatol*. **2005**, 124, 807-817.
58. Yu T.; MacPhail S.H.; Banath J.P.; Klokov D.; Olive P.L. Endogenous expression of phosphorylated histone H2AX in tumors in relation to DNA double-strand breaks and genomic instability. *DNA Repair* **2006**, 5, 935-946.
59. Risques R.A.; Lai L.A.; Brentnall T.A.; et al. Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. *Gastroenterology* **2008**, 135, 410-418.
60. Kao J.; Milano M.T.; Javaheri A.; Garofalo M.C,; Chmura S.J.; Weichselbaum R.R.; Kron S.J. Gamma-H2AX as a therapeutic target for improving the efficacy of radiation therapy. *Curr. Cancer Drug Targets* **2006**, 6, 197-205.
61. O´Connor M.J., Martin N.M.B.; Smith G.C.M. Targeted cancer therapies based on the inhibition of DNA strand break repair. *Oncogene* **2007**, 26, 7816-7824.
62. Martin N.M.B. DNA repair inhibition and cancer therapy. *J. Photochem. Photobiol.* **2001**, 63, 162-170.
63. Tichy A.; Muthna D.; Vavrova J.; Pejchal J.; et al. Caffeine-suppressed ATM pathway leads to decreased p53 phosphorylation and increased programmed cell death in gamma-irradiated leukemic MOLT-4 cells. *J. Appl. Biomed*. **2011**, 9, 49-56.
64. Hickson I.; Zhao Y.; Richardson C.J.; Green S.J.; Martin N.M.; et al. Identification and characterization of a novel and specific inhibitor of the Ataxia-telangiectasia mutated kinase ATM. *Cancer Res.* **2004**, 64, 9152-9159.
65. Zhao Y.; Huw D.T.; Batey M.A.; Cowell I.G.; Richardson C.J.; et al. Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. *Cancer Res.* **2006**, 66, 5354-5362.