Cancer Letters 327 (2012) 123–133

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Use of the γ-H2AX assay to monitor DNA damage and repair in translational cancer research

Alesia Ivashkevich [a], Christophe E. Redon [b], Asako J. Nakamura [c], Roger F. Martin [a], Olga A. Martin [a,d,*]

[a] Laboratory of Molecular Radiation Biology, Peter MacCallum Cancer Centre, Melbourne, Australia
[b] Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, Bethesda, USA
[c] Department of Anatomy and Cell Biology, Osaka Medical College, Osaka, Japan
[d] Department of Radiation Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia

ARTICLE INFO

Keywords:
γ-H2AX
Double-strand breaks (DSBs)
Translational research
Chemotherapy
Radiation therapy
Radiosensitivity
Biodosimetry
Imaging

ABSTRACT

Formation of γ-H2AX in response to DNA double stranded breaks (DSBs) provides the basis for a sensitive assay of DNA damage in human biopsies. The review focuses on the application of γ-H2AX-based methods to translational studies to monitor the clinical response to DNA targeted therapies such as some forms of chemotherapy, external beam radiotherapy, radionuclide therapy or combinations thereof. The escalating attention on radiation biodosimetry has also highlighted the potential of the assay including renewed efforts to assess the radiosensitivity of prospective radiotherapy patients. Finally the γ-H2AX response has been suggested as a basis for an *in vivo* imaging modality.

1. Introduction

The development of biomarkers to monitor and predict the efficacy of cancer chemo- and radiotherapy is a continuing endeavor in cancer therapy. Whilst macroscopic imaging can be useful, especially PET using ^{18}F-fluorodeoxyglucose [1], the response time deems these to be largely retrospective investigations of the response to therapy. The same can be said of metabolic markers, such as the classic example of prostate-specific antigen (PSA) [2]. Thus there is a rapidly progressing effort to develop short term response markers based on tissue specific gene and protein expression pro-

chemical response to this damage. However, until recently, the available methods were limited by poor sensitivity (relative to the size of available biopsy samples) and/or long turn-around time. Thus the emergence of a rapid, sensitive method to quantify the DNA damage that is apparent in cells shortly after treatment has great potential to monitor not only the response of the tumor, but also the effects of the treatment on "at risk" normal tissues, to thus assess therapeutic ratio. Assessment of DNA damage in normal tissues could have predictive value for both acute and long-term consequences of therapy.

In recent years, a new biomarker, the phosphorylated histone H2AX, has become a powerful tool to monitor DNA DSBs in translational cancer research [4]. H2AX, a variant of the core histone H2A family, contains a unique SQ motif within its C-terminal tail that is highly conserved from plants to human, suggesting a crucial role throughout evolution. In 1998, Dr. Bill Bonner's group at NIH first reported the phosphorylation of the omega-4 serine (Ser 139) in this SQ motif immediately after the introduction of DNA damage in human cells [5]. The phosphorylated form of H2AX was named γ-H2AX because it was first observed in cells exposed to γ-rays. Upon DSB induction, H2AX molecules are rapidly phosphorylated by PI3-kinases, such as ATM, ATR and DNA-PK, depending on the source of DNA damage and timing [4,6]. γ-H2AX following n minutes and reaches maximum levels after 30 min [7]. By using specific antibodies against γ-H2AX several important findings have been demonstrated. First, H2AX phosphorylation occurred in the chromatin surrounding a DSB site. Second, hundreds to thousands of γ-H2AX molecules surround one DSB to form a focus which may function both to open the chromatin structure and to serve as a platform for the accumulation of many factors involved in the DNA damage response [8,9].

Since its discovery, the γ-H2AX assay has been largely used for basic research to better understand the cellular mechanisms of different DNA damage repair pathways. In fact, many novel proteins involved in DNA damage response have been identified by their interaction and/or co-localization with γ-H2AX ([8,10–14] and Fig. 1). They sequentially assemble in a time-dependent manner [8,14]. The accumulation of repair and signaling factors is a facilitating and amplifying step of signal transduction and check-

* Corresponding author. Address: Department of Radiation Oncology, Peter MacCallum Cancer Centre, 12 St. Andrew's Place, East Melbourne, VIC 3002, Australia. Tel.: +61 3 9656 1357.
E-mail address: olga.martin@petermac.org (O.A. Martin).

0304-3835
doi:10.1016/j.canlet.2011.12.025

point pathways, and their retention and subsequent increase may be mediated through interactions between SQ motif and specific domains of repair/signaling proteins. Knock-out of H2AX causes a pleiotropic phenotype of radiation sensitivity, growth retardation, immunological deficiency and sterility of male mice. It is also associated with chromosomal instability and defective DSB repair [15]. As an adjunct to such basic studies, especially in the last 5 years, there has been an increasing use of γ-H2AX in translational studies to measure the biological effects of DNA damaging agents used in both chemotherapy and radiotherapy [16], including drug discovery and *in vitro* testing [17,18]. Indeed, this accelerating trend has prompted this review.

Given the nexus between DNA double-strand breaks (DSBs) and the γ-H2AX response, the use of the latter to monitor biologically important DNA damage invokes two assumptions that must be acknowledged. The first of these is that DSBs represent the only form of critical DNA damage. Whilst it is clearly established that a single unrepaired DSB can cause cell-killing, chromosome instability and gene mutation which may result in carcinogenesis [19], clustered oxidative DNA lesions (OCDLs) are also important. OCDLs (two or more oxidative DNA lesions generated within 10 base pairs of each other) can also result in detrimental cell consequences; they are difficult to repair and contribute to mutagenesis [20]. However, DSBs can be generated by the process of repairing OCDLs [21,22], so it seems reasonable to assume that these two types of lesions are not mutually exclusive. The second assumption, that exogenous DNA damaging agents are the only source of DSBs, is of more concern, and is addressed in Section 7.

Inspection of Fig. 1 raises the question as to why γ-H2AX has attracted so much attention, as well as the possibility that other repair proteins might provide a better basis for an assay of DNA damage. Indeed many such proteins, such as 53BP1, RAD50, MRE11, NBS1, phospho-ATM and many others can also be detected as repair foci. Part of the answer to this may simply reside in the historical momentum; the literature is dominated by γ-H2AX. However an important feature of γ-H2AX is that it is a new species induced by DSBs formation, whereas 53BP1 for example forms foci by translocation. Also, there is considerable evidence of the heterogeneity of foci formation by many other DNA damage repair proteins. Some, such as 53BP1, NBS1 and MRE11 are dissociated from DNA damage foci in the mitotic stage, whereas γ-H2AX foci are formed throughout the cell cycle [23–25]. Finally, γ-H2AX is a universal DSB marker. As mentioned above, SQ motif is highly conserved and H2AX phosphorylation occurs upon DNA damage induction across species [10]. This characteristic is quite advantageous and allows clinical researchers to use this biomarker to evaluate treatment efficacy in other species before translational applications.

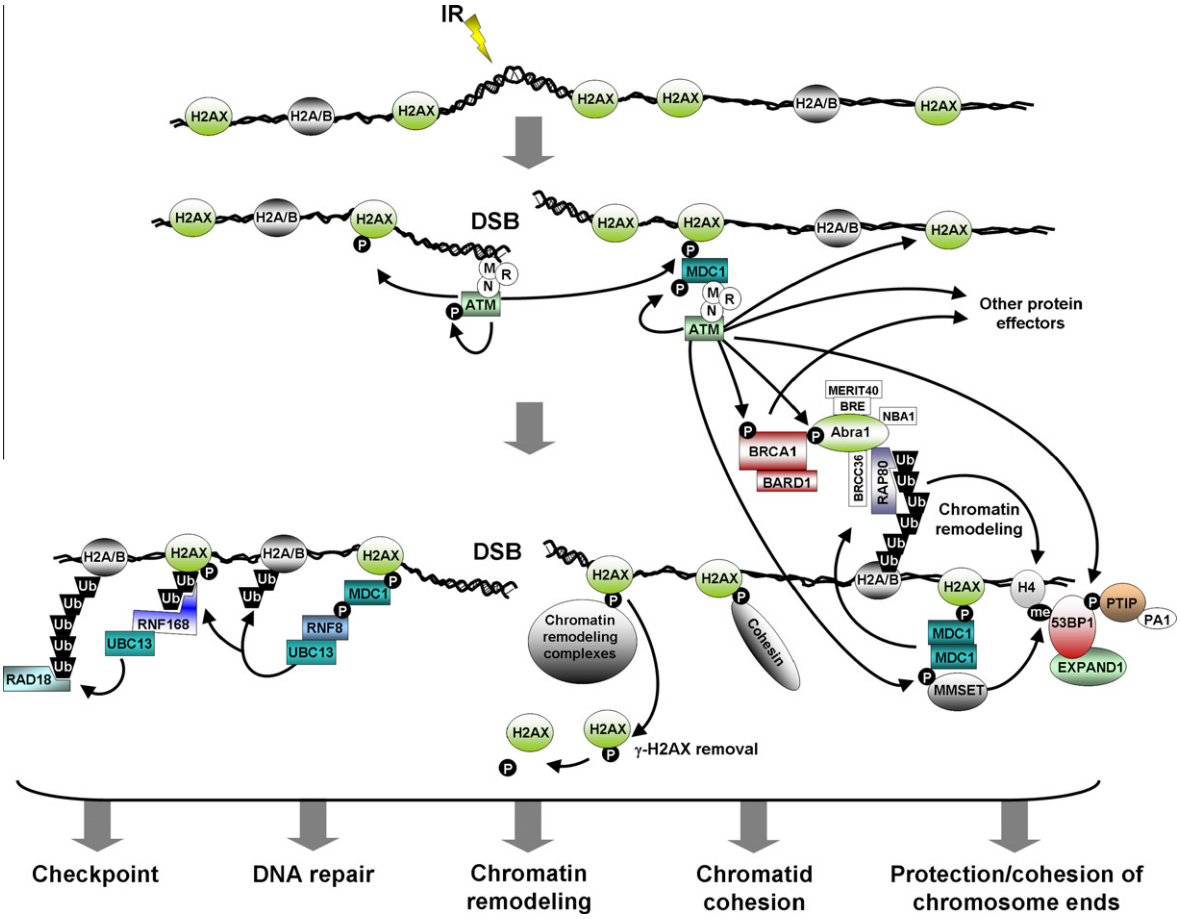

Fig. 1. γ-H2AX formation and its role in the irradiation-induced DNA repair foci. The foci assembly is a hierarchical process which starts with the DSB recognition by the MRN (MRE11-RAD50-NBS1) complex, recruitment of the ATM kinase and its autophosphorylation at the DSB site. The resulting ATM-mediated phosphorylation of H2AX (γ-H2AX) allows the recruitment of MDC1. MDC1 binding to γ-H2AX, in turn, allows the enrollment of other proteins including the MRN complex and ATM. Additional recruitment of ATM will then permit accrued phosphorylation of H2AX and other DNA repair proteins concentrating at the DSB (RNF8, BRCA1, 53BP1, etc.). MDC1 also recruits RNF8, an ubiquitin ligase which initiates histone H2 (H2AX, H2A, H2B) poly-ubiquitylation at DSB sites. These histone modifications allocate a second wave of protein accumulation, including proteins and/or protein complexes such as the BRCA1 A complex, 53BP1, RAD18, PTIP, EXPAND1, etc. γ-H2AX is also involved in the recruitment of chromatin remodeling complexes and chromatid cohesins. Foci formation is thought to stimulate DNA repair and checkpoint activation, to allow chromatin remodeling and sister chromatid cohesion and to facilitate cohesion of broken chromosome ends. P: phosphate, Ub: ubiquitin, me: methyl, M: MRE11, N: NBS1, R: RAD50. To simplify, single histones are shown.

This review focuses on the use of γ-H2AX assay to monitor both drug and irradiation responses in cancer patients as well as to improve clinical protocols. We also discuss the options available to clinicians for biospecimen sampling and the techniques employed for γ-H2AX analysis.

2. Assays for γ-H2AX detection and choice of biospecimens

The detection of γ-H2AX relies on immunological techniques using specific monoclonal and/or polyclonal antibodies against the H2AX C-terminal phosphorylated peptide CKATQAS(PO4)QEY [26]. Total γ-H2AX levels can be measured either in cell and/or tissue lysates or directly in cells and tissues [4]. In the first approach, the techniques establish the overall γ-H2AX levels in lysates by using immunoblotting or the enzyme-linked immunosorbent assay (ELISA) [27,28]. While immunoblotting is a useful tool for basic research, a lack of sensitivity generally makes this system unpractical for clinical samples. However, the high sensitivity of the ELISA recently allowed this technique to be developed for clinical trials [27]. In the second approach, γ-H2AX levels are measured directly in cell nuclei by microscopy or fluorescence-activated cell sorting (FACS). Several reasons make microscopy the method of choice to detect γ-H2AX levels in patients' samples (Tables 1 and 2). First, large numbers of γ-H2AX molecules formed at DNA break sites create bright foci that allow detection of individual DSBs making foci counting the most sensitive assay to detect DNA damage. Second, only microscopy reveals valuable information inaccessible by other methods, such as a differential response in tumor tissues (non-homogeneous *vs.* homogenous DNA damage induction) and the extent of DNA damage (separate foci in weakly damaged cells *vs.* pan-staining in suspected apoptotic cells).

The γ-H2AX assay has been applied for a variety of human tissues and cells. The best way to monitor the efficiency of cancer therapy would be to directly assay tumor biopsies [29], although a high variability of responses can occur due to cellular heterogeneity and vascularization. Another means to directly assay cancerous cells is by using circulating tumor cells (CTCs) [27]. Because CTCs have detached from primary or metastatic tumors, they circulate in the bloodstream and can be isolated from patients' blood samples [30]. Consequently, treatment leading to DNA damage in tumors can result in the release of CTCs bearing γ-H2AX-detectable DSBs. Wang et al. showed dynamic changes in CTC numbers in circulating blood, as well as increased γ-H2AX levels in cancer patients undergoing chemotherapy and demonstrated the promise to utilize such cells to monitor the effects of DNA damaging agents on tumors in future clinical trials [27].

However, in most cases collecting tumor samples is a challenging medical procedure, especially when repeated samplings are required. Therefore, clinicians often have to turn to safer, less invasive procedures that can be used routinely in the clinic, and will help evaluate the response to therapy as well as to ensure that reproducible results can be obtained. Cytotoxicity in normal tissues is an important side-effect of anti-cancer therapy, which can be monitored in tissues that can be obtained with minimal invasiveness.

Skin punch biopsies retain most of the skin layers including proliferating keratinocytes [31]. These tissues can be used for studies of the side-effects and efficiency of radiotherapy as well as the activity of drugs interfering with DNA metabolism [32].

Peripheral blood lymphocytes are the most common and easiest cells to obtain to analyze γ-H2AX formation *in vivo*. Under normal conditions (i.e., prior to DNA damage induction), lymphocytes show low γ-H2AX levels, typically less than one γ-H2AX focus per cell [33]. DSB induction leads to formation of large and bright γ-H2AX foci in response to as little as 1.2 mGy (equivalent to an average of 0.1 foci per cell) [34].

The γ-H2AX assay can also be performed in exfoliated oral epithelial (buccal) cells [35]. Exfoliative oral cytology has been previously advocated as a simple and non-invasive diagnostic technique [36,37]. Buccal cells are collected by mouthwash or scraping the patient's inner cheeks with a swab [35,38]. However, γ-H2AX quantification in such samples could be problematic as the collection of cells from the oral mucosa often leads to a highly heteroge-

Table 1
Non-exhaustive list of clinical studies using the γ-H2AX assay to measure the effects of chemotherapeutic drugs in cancer patients. The top of the table includes published clinical studies while the bottom part contains some studies obtained from the ClinicalTrials.gov database.

Tissues analyzed	Drug(s)	Condition	γ-H2AX detection	Phase	References
PBMCs	Clofarabine and cyclophosphamide	Refractory acute leukemias	FACS	I	[64]
PBMCs/tumor biopsies	SJG-136	Solid tumors	M	I	[65]
AML marrow blasts	Combination of tipifarnib and etoposide	AML	FACS	I	[64]
PBMCs	Combination of 5-azacytidine and entinostat	MDS, chronic myelomonocytic leukemia, and AML	I	I	[68]
Plucked eyebrows	Olaparib (AZD2281)	Breast cancer	M	I	[39]
CTCs/PBMCs	Combination of veliparib (ABT-888) with topotecan	Solid tumors and lymphomas	M	I	[67]
Tissues analyzed	Drug(s)	Condition	γ-H2AX detection	Phase	ClinicalTrials.gov identifier
ClinicalTrials.gov					
PBMCs, skin, hairs	Veliparib (ABT-888)	Breast, ovarian, pancreatic, prostate cancers; BRCA1, BRCA2 mutations carriers	N/S	I	NCT00892736
Tumor biopsies	7-t-butyldimethylsilyl-10-hydroxycamptothecin	Solid malignancies	M/I	I	NCT01202370
N/A	BSI-201 (Iniparib)	Advanced solid tumors	N/S	I	NCT01161836
N/A	Combination of BSI-201 and temozolomide	Glioblastoma	N/S	I, II	NCT00687765
N/A	BSI-201 (Iniparib)	Ovarian cancer	N/S	II	NCT01033123
N/A	TH-302	Glioma	M/I	II	NCT01403610
N/A	Combination of gemcitabine, carboplatin and BSI-201	Triple negative breast cancer	N/S	II	NCT00813956
N/A	Combination of gemcitabine/carboplatin plus BSI-201	Breast cancer	N/S	III	NCT00938652

Table 2
Non-exhaustive list of clinical studies using the γ-H2AX assay for various radiobiological applications: biodosimetry, radiosensitivity, prediction of the normal tissue side effects of radiotherapy alone or in combination with chemotherapy in cancer patients.

Tissues analyzed	Treatment	Condition, measured effects	γ-H2AX detection	References
Skin biopsy	0.05–1.1 Gy, first week of RT	Prostate cancer, normal tissue reactions	M	[32]
PBMCs	CT of benign or malignant neoplasms	Thorax and/or abdomen, biodosimetry	M	[81]
PBMCs	RT or RCT	Different tumors in different locations, biodosimetry	M	[72]
PBMCs	3D conformal- *vs.* step-and-shoot IMRT	Prostate cancer, biodosimetry	M	[85]
PBMCs	CT angiography	Biodosimetry	M	[82–84]
PBMCs	Radionuclide therapy (^{131}I)	Thyroid cancer, normal tissue toxicity	M	[96]
PBMCs	*Ex vivo* irradiation	Different tumors in different locations in children, radiosensitivity	M	[117]
PBMCs	60–66 Gy, 2 Gy/fraction, RT or RCT	Head-and-neck cancer, oral mucositis, radiosensitivity	M	[127]
PBMCs	*Ex vivo* irradiation	Cervix and endometrial cancer, late normal tissue reactions, radiosensitivity	M	[125]
PBMCs	*Ex vivo* irradiation	Head and neck cancer, acute normal tissue reactions, radiosensitivity	M	[126]
PBMCs	*Ex vivo* irradiation	Different tumors in different locations, late tissue reactions, radiosensitivity	FACS	[123]
T-cell lines	*Ex vivo* irradiation	ATM and NBS syndrome, radiosensitivity	M	[119]
Lymphocyte cell lines	*Ex vivo* irradiation	Different tumors in different locations, late tissue reactions, radiosensitivity	M	[114]

Abbreviations: M, microscopy; I, immunoblotting; FACS, fluorescence-activated cell sorting; CTCs, circulating tumor cells; PBMCs, peripheral blood mononuclear cells; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; N/S, not specified; CT, computed tomography; RT, radiotherapy; RCT, radiotherapy in combination with chemotherapy; ATM, ataxia telangiectasia; NBS, Nijmegen breakage syndrome.

neous mixture that may include enucleated, squamous and dead cells.

Finally, recent studies and clinical trials with cancer patients, as well as animal studies, show the potential of using plucked hairs to visualize γ-H2AX formation *in vivo* following exposure to both genotoxic drugs and radiation [39,40]. Samples can be obtained by plucking scalp and eyebrow hairs. A major advantage of the use of hair follicles rather than peripheral lymphocytes is that plucked hairs contain replicating cells that are more vulnerable to cancer drugs targeting DNA replication [41].

3. Development of high throughput systems for counting γ-H2AX foci

Although the most common approach is to manually count γ-H2AX foci either directly by eye with a microscope or in images previously captured, it is laborious, time consuming and a subject to a human error. Therefore, there is a clear incentive to develop high throughput foci counting systems for research and routine clinical assays. Validation of these automated systems unavoidably relies on comparisons with manual counting, but once achieved, enables extension to the analysis of foci size, and intensity. Moreover, the rapidity of automatic counting enables analysis of foci counts and other parameters amongst a population of cells much more feasible compared to manual counting.

To optimize the methods of γ-H2AX assessment and detection, number of studies came up with suggestions on the optimization of sample preparation [42], imaging modalities in cell cultures and in tissues [43–45], cytometric assessment of H2AX phosphorylation [46], automated microscopy and image processing [47,48], and led to the development of image analysis algorithms incorporated into specialized software, such as TGI [48], Image J [32,49–51], Image Pro Plus [52], Northern Eclipse [53], QuantityOne [54], AnalySis [55], Foci 8.0 [56], Histolab™ [57], Foci Counter [58], and others.

A detailed comparison of the available software is beyond the scope of this review, but some general notes of caution are appropriate. An automated foci counting requires thoughtful adjustment of the microscope settings, and analysis can be performed only on high quality images, taken with identical laser power and intensity, exposure and resolution settings. In addition, the resulting values are highly sensitive to the settings applied to the scored images, such as threshold, h-dom, and top hat. The values chosen for these settings can markedly impact on the outcome values. A simple illustration is that increase in threshold leads to lower foci counts. As with manual counting, the foci overlap can lead to their underscoring. A careful visual inspection of the derived foci and comparison to the original images is needed to define the optimal settings in automated high throughput image analysis. One of the most recently developed software, TGI, is an efficient program [48] for fast and reliable foci counting co-localization and intensity analysis. It includes the optimized automated nuclei identification for complex images of tissues that contain overlapping objects. The program enables automatic batch processing of a series of images.

Recent developments in automation have been incorporated into high throughput analysis platforms. The RABiT (Rapid Automated Biodosimetry Tool for Radiological Triage), a fully automated robotic system for sample preparation, staining, image acquisition and analysis, is currently under development at Columbia University, New York [59–62]. Designed to be used for high throughput biodosimetry, it explores the micronucleus assay for delayed time points and the γ-H2AX intensity (rather than foci counts) for detection of recent events. Although developed to handle very large numbers of samples for dosimetry screening in response to radiation incidents, such systems would be adaptable to routine clinical investigations.

4. γ-H2AX is a biomarker in cancer chemotherapy

Since its discovery, γ-H2AX has been extensively used in basic research in molecular pharmacology (see [18,63] for example), but its application as a pharmacodynamic marker in clinical studies is a relatively recent development [64]. In the last 4 years there has been a sharp increase in its use to monitor chemotherapy-induced DNA damage in cancer patients. Examples of the use of γ-H2AX in clinical studies are shown in Table 1 and can be found in clinicaltrials.gov. Since microscopy can discriminate between cells with low DNA damage (foci pattern) and cells potentially going through apoptosis (γ-H2AX panstaining), it is the preferred option for γ-H2AX analysis in patients' samples ([16] and Table 1). To date, there has been more than 35 clinical trials using γ-H2AX for evaluation of drug response in cancer patients. Of the four phases of clinical trials involved in the cancer drug-development process, there have been reports of the use of γ-H2AX as a bio-

marker in phases I, II and III studies. The phase I trials, designed to assess drug safety and pharmacokinetics, comprises more than half of total reported protocols using γ-H2AX. The γ-H2AX assay has been used to scrutinize the effects of various drugs that differ in the type of DNA damage they generate (topoisomerase I inhibitors, PARP inhibitors, DNA-alkylators, etc.). For example, in a phase I dose-escalation study aimed to establish the maximum tolerated dose of the sequence-selective minor groove DNA binding agent SJG-136 in patients with advanced solid tumors, evidence of drug–DNA interaction was shown through the formation of γ-H2AX foci in lymphocytes and tumor cells [65]. The practical use of the marker for pharmacodynamics was furthermore demonstrated in a phase I clinical trial after examination of γ-H2AX levels in another normal tissue – plucked eyebrow hair follicles from patients treated with a Poly(ADP-ribose) polymerases (PARP) inhibitor. This study showed a clear relationship between γ-H2AX levels and PARP inhibition [39]. Nevertheless, the majority of clinical protocols currently use γ-H2AX to investigate the efficacy of drug combinations. Such trials often combine a PARP inhibitor (i.e., olaparib and veliparib) with a DNA damaging agent (i.e., mitomycin C, carboplatin, cyclophosphamid, etc.). PARP inhibitors are compounds that specifically target the PARP-1, PARP-2 and tankyrase 1 (although PARP-1 is the most abundant PARP) (reviewed in [16]). PARP enzymes are strongly activated by both DNA single-strand breaks (SSBs) and DSBs, but because of their major contribution in DNA SSB repair, their inhibition has been proposed to lead to increased DSB formation [16]. For this reason, the use of PARP inhibitors (alone or combined with a DNA damaging agent leading to SSB) is a promising tool for killing tumors carrying defects in genes involved in DSB repair such as the *BRCA1* and *BRCA2* genes [66]. An illustration of the benefits of including the γ-H2AX assay in such protocols comes from a phase I study using ABT-888 (veliparib) in combination with topotecan hydrochloride, a topoisomerase I inhibitor in adults with lymphoma and refractory solid tumors [67]. Analysis of CTCs, tumors and mononucleated blood cells revealed increased γ-H2AX signals in patients receiving ABT-888 with topotecan. Thus, the studies proved that PARP inhibition can modulate the capacity to repair topoisomerase I-mediated DNA damage *in vivo* in cancer patients.

In a study of another drug combination, DNA methyltransferase inhibitors alone or in combination with histone deacetylase inhibitors which demonstrated clinical efficacy in patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), also resulted in increased γ-H2AX levels in peripheral blood lymphocytes [68].

Overall, while γ-H2AX is a newcomer in the array of clinical biomarkers, its use has become an important inclusion for most DNA damage repair studies. It is reasonable to think that because of its sensitivity, efficacy and convenience, more cancer clinical trials will take advantage of this marker in the future.

5. Biodosimetry

Although potentially a very generic term, "biodosimetry" is now commonly used in the context of retrospective estimation radiation dose from the extent of biological damage resulting from accidental radiation exposure; especially in scenarios when no physical dose estimate is available. Over the past decades, the most commonly used method in biological dosimetry was cytogenetic analysis, in which the stable (translocations) and unstable (dicentric chromosomes, micronuclei) chromosomal aberrations are counted in peripheral blood lymphocytes, along with a few other methods such as the glycophorin A mutation assay, electron paramagnetic resonance in tooth enamel, and the comet assay [69,70]. However, cytogenetic analysis is time-consuming; it implies growth stimulation for 48–72 h since chromosomal damage can only be measured in metaphases [71]. Thus, such an analysis is not suitable for the rapid identification of the most severely exposed individuals, which is required in the event of a large-scale radiation emergency, when reliable detection is needed for population triage during the first few hours after accidental radiation exposure.

Now the γ-H2AX assay has emerged as the preferred method. First, it has the advantage of the short time frame of the assay, and in the addition the high sensitivity; it can detect DNA damage induced by radiation doses as low as 1.2 mGy [34]. Measurement of DNA damage-induced γ-H2AX foci in lymphocytes avoids cell cycle effects since unstimulated lymphocytes are non-cycling cells. Many radiation accidents involve inhomogeneous exposures or partial body irradiation, and the γ-H2AX assay can be used to estimate the dose delivered to the whole body even if only a part of the body was irradiated, due to the redistribution of lymphocytes in blood flow [72,73].

Apart from retrospective evaluation of the absorbed dose in cases of accidental exposure to IR, and other scenarios such as in air and or space traveler [74], γ-H2AX-based biodosimetry has widespread clinical applications, summarized in Table 2. These applications involve patients undergoing radiological diagnostic or therapeutic treatments (CT scan, angioplasty, radiotherapy, etc.). There is similar potential in counterpart preclinical studies, as exemplified by the use of measurements of γ-H2AX levels in the development of a new micro-computed tomography-based conformal radiotherapy in a mouse model [75].

Interestingly, the numerous reports, include clinical studies, have established and validated the linear radiation dose response of the γ-H2AX assay [34,40,53,72,76–78], a necessary feature of a reliable biological dosimetry. For example, the linear correlation was shown between the numbers of γ-H2AX foci per lymphocyte obtained from peripheral blood samples and the integrated total body radiation dose in cancer patients treated with 3D conformal radiotherapy for a variety of tumor types [72], therefore making it possible to estimate the applied integral body dose, of course with consideration of the irradiation site and time dependence.

Ex vivo irradiation of freshly obtained lymphocytes resulted in induction of 10–15 foci per Gy [34,57,76], and no inter-individual heterogeneity was detected. However, high inter-individual variability was observed by Andrievski and Wilkins [77] which might be attributed to the applied dose of 10 Gy, as well as the use of FACS for quantification of H2AX phosphorylation. A number of studies have demonstrated that the γ-H2AX levels after *in vivo* exposure depend on the dose, exposed area of the body, and the duration/fractionation of the exposure. The γ-H2AX assay allowed demonstration of the localized irradiation of the mouse brain with CT-guided precision device [79]. The detailed study to characterize the *in vivo* formation and repair of DNA DSBs after CT examinations was performed by Lobrich et al. [80]. Interestingly, the study revealed that the repair kinetics of induced DNA damage appeared to be more efficient *in vivo* rather than *in vitro*. Phantom dosimetry-calculated total blood doses (13.85 mGy with whole-body CT and 5.16 mGy with chest CT) were confirmed by γ-H2AX-based dosimetrical estimations (16.4 mGy and 6.3 mGy) [81]. The studies of Kuefner et al. [82–84] adapted the γ-H2AX assay to assessment of DSB induction and repair in peripheral blood lymphocytes in patients undergoing angiographic procedures. A linear correlation was found between γ-H2AX kinetics and the radiation doses delivered during procedures; the assay has been considered to measure patients' individual repair capacity. Zwicker et al. [85] investigated the differences in dose delivery between SSIMRT and 3D treatment modalities of prostate cancer using γ-H2AX for biodosimetrical estimations. The results revealed a similarity between the γ-H2AX based measurements of the exposure of lymphocytes and physical dosimetry. Lower dose exposure of

lymphocytes was observed for the step-and-shoot IMRT compared to the 3D conformal treatment mode, and high dose exposure was the same in both cases. γ-H2AX measurements in skin biopsies taken from prostate cancer patients undergoing radiotherapy revealed a linear dose response and individual sensitivity to radiotherapy [32].

6. Monitoring effects of radionuclide therapy

The well-established phenomenon that decay of the Auger emitter ^{125}I, when incorporated into DNA, generates highly focused DNA damage [86] which corresponds to one DNA DSB per radioactive disintegration, and this has been used to correlate γ-H2AX foci with DSBs in cell cultures treated with ^{125}IdU. In S-phase synchronized cells, the data showed a direct correlation between the number of ^{125}I decays/DSBs and the numbers of foci per cell [87]. Studies with DNA binding ligands labeled with ^{125}I demonstrated that DNA breaks can be induced by Auger decay non-covalently associated with DNA with subsequent cytotoxicity, initially with intercalating ligands [88,89], and subsequently with minor groove-binders [90,91]. This prompted the strategy to deliver Auger-induced damage to genomic targets by triplex-forming oligonucleotides (TFOs) that bind specifically to their target sequences by forming hydrogen bonds within the major groove of the target duplex. This approach has been named antigene radiotherapy [92]. The γ-H2AX assay has been used to screen the wide variety of possible TFO modifications and proved to be useful for evaluation of cellular DNA accessibility of ^{125}I-labeled DNA targeting agents [93,94].

The γ-H2AX assay has also been used to monitor formation and persistence of DNA damage and cytotoxicity in human cancer cells produced by another Auger emitter, ^{111}In, that has been delivered to cell nuclei by pharmaceuticals that target human epidermal growth factor receptor (EGFR)-positive cancers and HER2/neu-amplified breast cancers [49,50]. In another study, γ-H2AX levels increased in cultured tumor cells confirming the genotoxic effect of radioimmunotherapy with alpha-particle emitting ^{213}Bi. The anti-tumor efficacy of this therapy was confirmed in a mouse model [95]. However, in a real clinical situation, during radionuclide therapy, directly targeted tumors are often not accessible; rather, normal tissue response to a circulating radionuclide (direct or bystander) can be monitored.

Radionuclide-induced DNA damage induction and repair kinetics *in vivo* can be very complex due to several factors. First, many of radionuclides used for therapy emit photons, heavy ion particles, and electrons; and collectively represent a wide range of intensity and distribution (within cells and at an organ level) of radiochemical damage. Second, the pharmacokinetics of radionuclide exposure is determined by the biochemical carrier and hence intracellular location. Third, the time course of radiation delivery is determined by the physical rate of exponential decay of a radioisotope as well as the pharmacokinetics of the carrier. Finally, there can be variations in radiosensitivity between patients, and also for particular patients, there can be different responses to consecutive rounds of treatment. In fact, the only *in vivo* study reported that the formation, persistence and disappearance of γ-H2AX and 53BP1 foci in mononuclear blood cells of patients with differentiated thyroid cancer after ^{131}I therapy varied dramatically between individuals [96]. Monitoring of this normal tissue response in parallel with the clinical tumor outcome, could also form a basis for routine monitoring of individual radiosensitivity to the therapy.

7. Individual radiosensitivity

Another important potential application of γ-H2AX assay is the assessment of radiosensitivity of prospective patients. Radiation therapy is the major cancer treatment modality either alone or in combination with chemotherapy or surgery. However, in some cases it can induce severe side effects due to normal tissue toxicity. In most schedules of radical radiotherapy aiming for local tumor control, the dose prescribed results in ~5% of patients experiencing severe side-effects. If the radiosensitive subpopulation could be identified in advance, customized radiotherapy treatments could avoid the severe side effects of the subpopulation, and enable safe escalation of dose in the majority of patients with normal radiosensitivity. The clinical challenge is not new, and many studies have addressed the necessity of developing the rapid, reliable and functional assays to predict the individual radiosensitivity [97–99].

The radiosensitive patients show pronounced clinical radiation reactions, such as acute effects (erythema, edema, mucositis, dry or moist desquamation) and late effects (telangiectasia, fibrosis, cancer induction, neurological effects and others). Up to 70% of cases of increased radiosensitivity remain unexplained, and individual differences in intrinsic cellular radiosensitivity, resulting from genetic variations and epigenetic factors might contribute to their occurrence [100].

Radiosensitivity is often observed in patients with genetic disorders associated with a defect in the DNA damage response and cell death pathways (reviewed in [101–103]). Among these are such well characterized syndromes as AT, AT-like disorder, Nijmegen breakage syndrome (NBS), Fanconi anemia (FA), Ligase IV syndrome, Seckel syndrome, Li-Fraumeni syndrome and familial retinoblastoma. Pollard et al. [104] reviewed 32 cases of over-reacting individuals, half of them were FA patients, who developed adverse reactions in response to radiotherapy. Most of them died within several months after radiation exposure took place. In addition to higher incidence of cancer and increased susceptibility to radiation, they are often associated with defects in immune and neurological systems. So far about 150 DNA repair genes have been identified, and the real number is probably higher, since the function of a significant part of known and putative genes in human genome has not been described yet [105,106].

Radiosensitivity is associated with deficiency in important cell functions, such as cell survival, DNA repair capacity, formation of chromosomal aberrations, and induction of cell death [107]. Therefore, *in vitro* measurements of cell functions have been used as endpoints to evaluate individual radiosensitivity. Several assays were tested for their potential to predict radiosensitivity by measuring DNA repair capacity, induction of cell death and cytogenetic effects, and ability of cell to survive and retain their proliferative capacity after irradiation [107]. Chromosomal aberrations (dicentrics and translocations) and clonogenic survival were shown to be able to predict intrinsic radiosensitivity [108–110]. However, these assays require cell activation or transformation, and the time required to produce the data varied from days in case of cytogenetic tests in lymphocytes (dysenteric chromosomes, micronuclei, translocations, DNA fragments) to months for clonogenic survival. It has been reported that transformation itself may disrupt the radiosensitivity of normal cells [111,112]. Geara et al. [109,113] reported lack of correlation between radiosensitivity of transformed lymphocytes and primary fibroblasts cultured from skin biopsy samples. Also, a recent study by Vasireddy et al. [114] reports a lack of correlation between radiosensitivity of transformed lymphocytes and clinical radiosensitivity.

The γ-H2AX assay is a quick read-out of DNA damage, can be performed on primary untransformed cells and thus presents a new opportunity. Whether it can be a surrogate for cytogenetic and clonogenic assays or should be considered independently is under investigation. Scoring dicentrics and quantification of γ-H2AX foci have been performed in lymphocytes of blood samples exposed to a CT scanner [115]. Although they both are appropriate methods to detect irradiation-induced damage, they quantify dif-

ferent steps of molecular modification of this damage. Whereas γ-H2AX foci resolve within several hours, resulting chromosomal changes can be present for long time forming a base for normal tissue toxicity, and can be quantified by scoring of chromosome aberrations. However, the number of residual γ-H2AX foci at 12 or 24 h post-irradiation was found to correlate with clonogenic survival and to predict differences in the radiation dose response relationship *in vitro* [56,116].

Addressing the question whether the γ-H2AX post-exposure kinetics correlates with clinically relevant endpoints evoked several recent studies. The γ-H2AX screen of lymphocytes from children with solid cancers identified three patients with impaired DSB repair capacities, and two of these repair deficient children developed acute normal tissue toxicities in response to DNA damaging cancer therapy [117]. Lobrich et al. [80] examined γ-H2AX induction in CT patients, and identified impaired kinetics of γ-H2AX foci in one radiosensitive patient. Analysis of γ-H2AX foci in cell lines derived from patients with severe or mild mutations in a core non-homologous end joining (NHEJ) factor, or mutations in ATM signaling pathway or Artemis allowed the identification of distinct typical DSB repair profiles associated with defects in early, late, or both early and late rejoining [118]. In mouse strains with various DSB repair deficiencies, even slight genetic impairments were reflected by the γ-H2AX assay in lymphocytes and tissues after total body irradiation [45].

The assay has already successfully identified AT, NBS, xeroderma pigmentosum, FA, retinoblastoma, Ligase IV syndrome patients and others [114,119–122]. Recently, the new translational opportunities of the γ-H2AX assay attracted attention of several international groups, but the reported results are somewhat contradictory. One study reports successful identification of radiosensitive patients based on their lower response to 1 Gy irradiation in primary lymphocytes and maintaining this unrepaired damage for longer time (12 out of 12 radiosensitive patients exhibited repair delay) [123], while another study suggests that low doses are the most effective for the difference detection [124]. However, other reports failed to prove the usefulness of this assay. Werbrouck et al. [125] found no difference in foci kinetics in irradiated *ex vivo* T-lymphocytes between the non to mild and moderate to severe late normal tissue reactions in gynaecological cancer patients, nor was there any correlation between the assay and acute normal tissue reactions during IMRT treatment in head and neck cancer patients [126]. The study of Fleckenstein et al. [127] did not reveal the difference between grade 3 and grade 2 mucositis with γ-H2AX foci counts estimated 24 h after radiation exposure, as well as DSB repair half times, although the authors established that the patients with an increased unrepaired DSBs after 24 h had an increased incidence of severe oral mucositis.

The apparent controversy as to the reliability of the γ-H2AX assay in assessing individual radiosensitivity prompts consideration of the importance of the cell cycle in the assay endpoint. Progression of damaged cells through the cell cycle can lead to further breakage in S-phase [118,128] and thus S-phase cells should be avoided for analysis. The recommended sample tissue, peripheral blood lymphocytes, are quiescent unless activated, and therefore, avoids interference by replication-associated DNA damage. Similarly, culture of fibroblasts taken as biopsy induces their accumulation in G0/G1 phases of the cell cycle by contact inhibition, therefore solving a problem of the contribution of S phase cells. However, the fact that homologous recombination (HR) only occurs in proliferating cells, imposes a dilemma for detection of radiosensitivity due to effects in HR. A combination of assays involving both non-proliferating and proliferating tissues (e.g. non-activated and activated lymphocytes) might be necessary to address this issue. Finally, it should be acknowledged that the relevance of lymphocyte-based assay to the complex process involved in late radiation toxicity has been questioned [129].

Therefore, whilst the overall simplicity and high sensitivity is attractive, the specificity of the method as a predictive assay for identification of radiosensitive patients is still under investigation. At this stage, further validation of the assay is required. However, even the correlation between the assay and clinical radiosensitivity is incomplete, the ability of the assay to detect that subset of radiosensitive patients with defective DNA DSB repair pathways would be valuable *per se*.

8. γ-H2AX -based imaging

Since anti-tumor treatments are aimed to kill tumors with minimal side effects to normal cells, direct imaging of DNA damage *in vivo* has a great potential for monitoring therapeutic efficacy and patients' responses to chemo- and radiotherapy. Such *in vivo* imaging would also have a great diagnostic as well as prognostic advantage if increased DNA damage levels in various organs associated with genome instability, chronic inflammations and early carcinogenesis [130–134] are identified.

Until recently, quantification of DNA damage *in vivo* was not possible; rather, an approach to measure apoptosis *in vivo* has been developed. Annexin V, a protein with high activity to apoptotic cells, has been labeled with different tags, fluorochromes and radionuclides [135–138]. Development of small amphipathic molecules accumulating in apoptotic cells is another approach [139]. Edgington et al. [138] describe the use of fluorescently-labeled probes conjugated with cell-permeable peptide sequence, that covalently label activated caspases in mice with induced apoptosis. However, apoptosis in tumors is not the only outcome of anti-cancer therapy; other processes such as necrosis, mitotic catastrophe, autophagy and senescence also play a role.

Since DNA DSBs are involved in multiple biological processes including apoptosis, imaging of DSBs *in vivo* would be much more informative. However, DSB levels are generally low (difficult target for imaging), and they are not easy to access because of their nuclear location. Two very recent studies explore the opportunity to directly visualize DSBs *in vivo*. Detection of the γ-H2AX signal amplified at the sites of DSBs in a megabase region [4] is the strategy to overcome their low numbers. Cornelissen et al. [140] developed a strategy to detect γ-H2AX *in vivo* by a cell-penetrating Tat peptide containing a nuclear localization signal (NLS) sequence. When this peptide is attached to a fluorophore- or ^{111}In-labeled anti-γ-H2AX antibody, it labels phosphorylated H2AX in live cells and mice following DNA damage.

A quite different strategy described in [141] is based on the fact that γ-H2AX is physically associated with MDC1 DSB repair protein. Thus, N- and C-terminal fragments of firefly luciferase genes were fused with H2AX and MDC1 genes, respectively. In live mammalian cells, upon a DSB formation, H2AX is phosphoryated and associates with the MDC1 bringing together N- and C-luciferase fragments and reconstituting luciferase activity. Although this innovative approach is peripheral to translational oncology, it has revealed new radiobiological information. For example, the authors detected a second wave of DSB induction in irradiated transplanted tumors days after radiation exposure *in vivo,* in addition to the initial rapid induction of DSBs.

9. Conclusions

The widespread versatility and potential of the γ-H2AX assay for translational studies stem mainly from two features of the assay. Firstly, its sensitivity enables quantification of the biological effects of low doses particularly in the context of radiation biodos-

imetry (as well as the effects of low concentrations of genotoxic drugs and higher doses of irradiation in cancer chemo- and radiotherapy); also the assay only requires a small sample of biological material. Secondly the very short response time for the development of the endpoint following induction of a DNA DSB enables many new opportunities. Amongst these is the response of untransformed lymphocytes to *ex vivo* irradiation as a means of assessing radiosensitivity. Also the assay is amenable to automation. All these features will ensure a continuing escalation of the application of the γ-H2AX assay, particularly in translational studies.

Acknowledgements

This study was partly funded by the Intramural Research Program of the National Cancer Institute, National Institutes of Health and by the NIAID Radiation/Nuclear Countermeasures Program.

References

[1] M.P. MacManus, R.J. Hicks, Where do we draw the line? Contouring tumors on positron emission tomography/computed tomography, International Journal of Radiation Oncology, Biology, Physics 71 (2008) 2–4.
[2] J.M. Croswell, B.S. Kramer, E.D. Crawford, Screening for prostate cancer with PSA testing: current status and future directions, Oncology (Williston Park) 25 (2011) 452–460. 463.
[3] M. Sikaroodi, Y. Galachiantz, A. Baranova, Tumor markers: the potential of "omics" approach, Current Molecular Medicine 10 (2010) 249–257.
[4] W.M. Bonner, C.E. Redon, J.S. Dickey, A.J. Nakamura, O.A. Sedelnikova, S. Solier, Y. Pommier, gammaH2AX and cancer, Nature Reviews Cancer 8 (2008) 957–967.
[5] E.P. Rogakou, D.R. Pilch, A.H. Orr, V.S. Ivanova, W.M. Bonner, DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139, The Journal of Biological Chemistry 273 (1998) 5858–5868.
[6] A. Kinner, W. Wu, C. Staudt, G. Iliakis, {gamma}-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin, Nucleic Acids Research (2008).
[7] E.P. Rogakou, C. Boon, C. Redon, W.M. Bonner, Megabase chromatin domains involved in DNA double-strand breaks in vivo, The Journal of Cell Biology 146 (1999) 905–916.
[8] T.T. Paull, E.P. Rogakou, V. Yamazaki, C.U. Kirchgessner, M. Gellert, W.M. Bonner, A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage, Current Biology 10 (2000) 886–895.
[9] A. Celeste, O. Fernandez-Capetillo, M.J. Kruhlak, D.R. Pilch, D.W. Staudt, A. Lee, R.F. Bonner, W.M. Bonner, A. Nussenzweig, Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks, Nature Cell Biology 5 (2003) 675–679.
[10] C. Redon, D. Pilch, E. Rogakou, O. Sedelnikova, K. Newrock, W. Bonner, Histone H2A variants H2AX and H2AZ, Current Opinion in Genetics and Development 12 (2002) 162–169.
[11] O.A. Sedelnikova, D.R. Pilch, C. Redon, W.M. Bonner, Histone H2AX in DNA damage and repair, Cancer Biology and Therapy 2 (2003) 233–235.
[12] D.R. Pilch, O.A. Sedelnikova, C. Redon, A. Celeste, A. Nussenzweig, W.M. Bonner, Characteristics of gamma-H2AX foci at DNA double-strand breaks sites, Biochemistry and Cell Biology 81 (2003) 123–129.
[13] O. Fernandez-Capetillo, A. Celeste, A. Nussenzweig, Focusing on foci: H2AX and the recruitment of DNA-damage response factors, Cell Cycle 2 (2003) 426–427.
[14] M. Stucki, J.A. Clapperton, D. Mohammad, M.B. Yaffe, S.J. Smerdon, S.P. Jackson, MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks, Cell 123 (2005) 1213–1226.
[15] A. Celeste, S. Petersen, P.J. Romanienko, O. Fernandez-Capetillo, H.T. Chen, O.A. Sedelnikova, B. Reina-San-Martin, V. Coppola, E. Meffre, M.J. Difilippantonio, C. Redon, D.R. Pilch, A. Olaru, M. Eckhaus, R.D. Camerini-Otero, L. Tessarollo, F. Livak, K. Manova, W.M. Bonner, M.C. Nussenzweig, A. Nussenzweig, Genomic instability in mice lacking histone H2AX, Science 296 (2002) 922–927.
[16] C.E. Redon, A.J. Nakamura, Y.W. Zhang, J.J. Ji, W.M. Bonner, R.J. Kinders, R.E. Parchment, J.H. Doroshow, Y. Pommier, Histone gammaH2AX and poly(ADP-ribose) as clinical pharmacodynamic biomarkers, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 16 (2010) 4532–4542.
[17] K. Takagi, T.S. Dexheimer, C. Redon, O. Sordet, K. Agama, G. Lavielle, A. Pierre, S.E. Bates, Y. Pommier, Novel E-ring camptothecin keto analogues (S38809 and S39625) are stable, potent, and selective topoisomerase I inhibitors without being substrates of drug efflux transporters, Molecular Cancer Therapeutics 6 (2007) 3229–3238.
[18] K.A. Bridges, H. Hirai, C.A. Buser, C.S. Brooks, H. Liu, T.A. Buchholz, J.M. Molkentine, K.A. Mason, R.E. Meyn, MK-1775, a novel wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research (2011).
[19] D. Ziech, R. Franco, A. Pappa, M.I. Panayiotidis, Reactive oxygen species (ROS) – induced genetic and epigenetic alterations in human carcinogenesis, Mutation Research 711 (2011) 167–173.
[20] S. Nowsheen, R.L. Wukovich, K. Aziz, P.T. Kalogerinis, C.C. Richardson, M.I. Panayiotidis, W.M. Bonner, O.A. Sedelnikova, A.G. Georgakilas, Accumulation of oxidatively induced clustered DNA lesions in human tumor tissues, Mutation Research 674 (2009) 131–136.
[21] A.G. Georgakilas, Processing of DNA damage clusters in human cells: current status of knowledge, Molecular Biosystems 4 (2008) 30–35.
[22] A. Georgakilas, Detection of clustered DNA lesions: biological and clinical applications, World Journal of Biological Chemistry 2 (2011) 173–176.
[23] S. Giunta, R. Belotserkovskaya, S.P. Jackson, DNA damage signaling in response to double-strand breaks during mitosis, The Journal of Cell Biology 190 (2010) 197–207.
[24] A.J. Nakamura, V.A. Rao, Y. Pommier, W.M. Bonner, The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks, Cell Cycle 9 (2010) 389–397.
[25] G. Nelson, M. Buhmann, T. von Zglinicki, DNA damage foci in mitosis are devoid of 53BP1, Cell Cycle 8 (2009) 3379–3383.
[26] J.S. Dickey, C.E. Redon, A.J. Nakamura, B.J. Baird, O.A. Sedelnikova, W.M. Bonner, H2AX: functional roles and potential applications, Chromosoma 118 (2009) 683–692.
[27] L.H. Wang, T.D. Pfister, R.E. Parchment, S. Kummar, L. Rubinstein, Y.A. Evrard, M.E. Gutierrez, A.J. Murgo, J.E. Tomaszewski, J.H. Doroshow, R.J. Kinders, Monitoring drug-induced gammaH2AX as a pharmacodynamic biomarker in individual circulating tumor cells, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 16 (2010) 1073–1084.
[28] K. Matsuzaki, A. Harada, A. Takeiri, K. Tanaka, M. Mishima, Whole cell-ELISA to measure the gammaH2AX response of six aneugens and eight DNA-damaging chemicals, Mutation Research 700 (2010) 71–79.
[29] K. Dellas, M. Bache, S.U. Pigorsch, H. Taubert, M. Kappler, D. Holzapfel, E. Zorn, H.J. Holzhausen, G. Haensgen, Prognostic impact of HIF-1alpha expression in patients with definitive radiotherapy for cervical cancer, Strahlentherapie und Onkologie: Organ der Deutschen Rontgengesellschaft .. [et al] 184 (2008) 169–174.
[30] E.S. Lianidou, A. Markou, Circulating tumor cells in breast cancer: detection systems, molecular characterization, and future challenges, Clinical Chemistry (2011).
[31] S. Papini, D. Cecchetti, D. Campani, W. Fitzgerald, J.C. Grivel, S. Chen, L. Margolis, R.P. Revoltella, Isolation and clonal analysis of human epidermal keratinocyte stem cells in long-term culture, Stem Cells 21 (2003) 481–494.
[32] O.F. Qvarnstrom, M. Simonsson, K.A. Johansson, J. Nyman, I. Turesson, DNA double strand break quantification in skin biopsies, Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology 72 (2004) 311–317.
[33] D. Geisel, J.T. Heverhagen, M. Kalinowski, H.J. Wagner, DNA double-strand breaks after percutaneous transluminal angioplasty, Radiology 248 (2008) 852–859.
[34] K. Rothkamm, M. Lobrich, Evidence for a lack of DNA double-strand break repair in human cells exposed to very low X-ray doses, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 5057–5062.
[35] A.J. Yoon, J. Shen, H.C. Wu, C. Angelopoulos, S.R. Singer, R. Chen, R.M. Santella, Expression of activated checkpoint kinase 2 and histone 2AX in exfoliative oral cells after exposure to ionizing radiation, Radiation Research 171 (2009) 771–775.
[36] L.A. Cabral, C.F. Lima, M.L. Coutinho de Oliveira, A.A. Brandao, J.D. Almeida, Oral exfoliative cytology for the diagnosis of paracoccidoidomycosis in a patient with human immunodeficiency virus: a case report, Acta Cytologica 54 (2010) 1127–1129.
[37] M. Perez-Sayansm, J.M. Somoza-Martin, F. Barros-Angueira, M.D. Reboiras-Lopez, P. Gandara-Vila, J.M. Gandara Rey, A. Garcia-Garcia, Exfoliative cytology for diagnosing oral cancer, Biotechnic and Histochemistry: Official Publication of the Biological Stain Commission 85 (2010) 177–187.
[38] J.E. Gonzalez, S.H. Roch-Lefevre, T. Mandina, O. Garcia, L. Roy, Induction of gamma-H2AX foci in human exfoliated buccal cells after in vitro exposure to ionising radiation, International Journal of Radiation Biology 86 (2010) 752–759.
[39] P.C. Fong, D.S. Boss, T.A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, P. Mortimer, H. Swaisland, A. Lau, M.J. O'Connor, A. Ashworth, J. Carmichael, S.B. Kaye, J.H. Schellens, J.S. de Bono, Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers, The New England Journal of Medicine 361 (2009) 123–134.
[40] C.E. Redon, A.J. Nakamura, K. Gouliaeva, A. Rahman, W.F. Blakely, W.M. Bonner, The use of gamma-H2AX as a biodosimeter for total-body radiation exposure in non-human primates, PloS One 5 (2010) e15544.
[41] C.G. Gho, J.E. Braun, C.M. Tilli, H.A. Neumann, F.C. Ramaekers, Human follicular stem cells: their presence in plucked hair and follicular cell culture, The British Journal of Dermatology 150 (2004) 860–868.
[42] I.H. Ismail, T.I. Wadhra, O. Hammarsten, An optimized method for detecting gamma-H2AX in blood cells reveals a significant interindividual variation in the gamma-H2AX response among humans, Nucleic Acids Research 35 (2007) e36.

[43] B. Gavrilov, I. Vezhenkova, D. Firsanov, L. Solovjeva, M. Svetlova, V. Mikhailov, N. Tomilin, Slow elimination of phosphorylated histone gamma-H2AX from DNA of terminally differentiated mouse heart cells in situ, Biochemical and Biophysical Research Communications 347 (2006) 1048–1052.
[44] N. Bhogal, F. Jalali, R.G. Bristow, Microscopic imaging of DNA repair foci in irradiated normal tissues, International Journal of Radiation Biology 85 (2009) 732–746.
[45] C.E. Rube, S. Grudzenski, M. Kuhne, X. Dong, N. Rief, M. Lobrich, C. Rube, DNA double-strand break repair of blood lymphocytes and normal tissues analysed in a preclinical mouse model: implications for radiosensitivity testing, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 14 (2008) 6546–6555.
[46] X. Huang, Z. Darzynkiewicz, Cytometric assessment of histone H2AX phosphorylation: a reporter of DNA damage, Methods in Molecular Biology 314 (2006) 73–80.
[47] Y.N. Hou, A. Lavaf, D. Huang, S. Peters, R. Huq, V. Friedrich, B.S. Rosenstein, J. Kao, Development of an automated gamma-H2AX immunocytochemistry assay, Radiation Research 171 (2009) 360–367.
[48] A.N. Ivashkevich, O.A. Martin, A.J. Smith, C.E. Redon, W.M. Bonner, R.F. Martin, P.N. Lobachevsky, gammaH2AX foci as a measure of DNA damage: a computational approach to automatic analysis, Mutation Research 711 (2011) 49–60.
[49] K.E. Bailey, D.L. Costantini, Z. Cai, D.A. Scollard, Z. Chen, R.M. Reilly, K.A. Vallis, Epidermal growth factor receptor inhibition modulates the nuclear localization and cytotoxicity of the Auger electron emitting radiopharmaceutical 111In-DTPA human epidermal growth factor, Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine 48 (2007) 1562–1570.
[50] D.L. Costantini, C. Chan, Z. Cai, K.A. Vallis, R.M. Reilly, (111)In-labeled trastuzumab (Herceptin) modified with nuclear localization sequences (NLS): an Auger electron-emitting radiotherapeutic agent for HER2/neu-amplified breast cancer, Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine 48 (2007) 1357–1368.
[51] Z. Cai, K.A. Vallis, R.M. Reilly, Computational analysis of the number, area and density of gamma-H2AX foci in breast cancer cells exposed to (111)In-DTPA-hEGF or gamma-rays using Image-J software, International Journal of Radiation Biology 85 (2009) 262–271.
[52] W. Bocker, G. Iliakis, Computational methods for analysis of foci: validation for radiation-induced gamma-H2AX foci in human cells, Radiation Research 165 (2006) 113–124.
[53] S.H. MacPhail, J.P. Banath, T.Y. Yu, E.H. Chu, H. Lambur, P.L. Olive, Expression of phosphorylated histone H2AX in cultured cell lines following exposure to X-rays, International Journal of Radiation Biology 79 (2003) 351–358.
[54] E.L. Leatherbarrow, J.V. Harper, F.A. Cucinotta, P. O'Neill, Induction and quantification of gamma-H2AX foci following low and high LET-irradiation, International Journal of Radiation Biology 82 (2006) 111–118.
[55] H. Mahrhofer, S. Burger, U. Oppitz, M. Flentje, C.S. Djuzenova, Radiation induced DNA damage and damage repair in human tumor and fibroblast cell lines assessed by histone H2AX phosphorylation, International Journal of Radiation Oncology, Biology, Physics 64 (2006) 573–580.
[56] E. Markova, N. Schultz, I.Y. Belyaev, Kinetics and dose-response of residual 53BP1/gamma-H2AX foci: co-localization, relationship with DSB repair and clonogenic survival, International Journal of Radiation Biology 83 (2007) 319–329.
[57] S. Roch-Lefevre, T. Mandina, P. Voisin, G. Gaetan, J.E. Mesa, M. Valente, P. Bonnesoeur, O. Garcia, L. Roy, Quantification of gamma-H2AX foci in human lymphocytes: a method for biological dosimetry after ionizing radiation exposure, Radiation Research 174 (2010) 185–194.
[58] A. Jucha, A. Wegierek-Ciuk, Z. Koza, H. Lisowska, A. Wojcik, M. Wojewodzka, A. Lankoff, FociCounter: a freely available PC programme for quantitative and qualitative analysis of gamma-H2AX foci, Mutation Research (2009).
[59] G. Garty, Y. Chen, A. Salerno, H. Turner, J. Zhang, O. Lyulko, A. Bertucci, Y. Xu, H. Wang, N. Simaan, G. Randers-Pehrson, Y.L. Yao, S.A. Amundson, D.J. Brenner, The RABIT: a rapid automated biodosimetry tool for radiological triage, Health Physics 98 (2010) 209–217.
[60] G. Garty, Y. Chen, H.C. Turner, J. Zhang, O.V. Lyulko, A. Bertucci, Y. Xu, H. Wang, N. Simaan, G. Randers-Pehrson, Y. Lawrence Yao, D.J. Brenner, The RABiT: a rapid automated biodosimetry tool for radiological triage. II. Technological developments, International Journal of Radiation Biology 87 (2011) 776–790.
[61] H.C. Turner, D.J. Brenner, Y. Chen, A. Bertucci, J. Zhang, H. Wang, O.V. Lyulko, Y. Xu, I. Shuryak, J. Schaefer, N. Simaan, G. Randers-Pehrson, Y.L. Yao, S.A. Amundson, G. Garty, Adapting the gamma-H2AX assay for automated processing in human lymphocytes. 1. Technological aspects, Radiation Research (2010).
[62] H.C. Turner, D.J. Brenner, Y. Chen, A. Bertucci, J. Zhang, H. Wang, O.V. Lyulko, Y. Xu, I. Shuryak, J. Schaefer, N. Simaan, G. Randers-Pehrson, Y.L. Yao, S.A. Amundson, G. Garty, Adapting the gamma-H2AX assay for automated processing in human lymphocytes. 1. Technological aspects, Radiation Research 175 (2011) 282–290.
[63] T.J. Guzi, K. Paruch, M.P. Dwyer, M. Labroli, F. Shanahan, N. Davis, L. Taricani, D. Wiswell, W. Seghezzi, E. Penaflor, B. Bhagwat, W. Wang, D. Gu, Y. Hsieh, S. Lee, M. Liu, D. Parry, Targeting the replication checkpoint using SCH 900776, a potent and functionally selective CHK1 inhibitor identified via high content screening, Molecular Cancer Therapeutics 10 (2011) 591–602.
[64] J.E. Karp, R.M. Ricklis, K. Balakrishnan, J. Briel, J. Greer, S.D. Gore, B.D. Smith, M.A. McDevitt, H. Carraway, M.J. Levis, V. Gandhi, A phase 1 clinical-laboratory study of clofarabine followed by cyclophosphamide for adults with refractory acute leukemias, Blood 110 (2007) 1762–1769.
[65] D. Hochhauser, T. Meyer, V.J. Spanswick, J. Wu, P.H. Clingen, P. Loadman, M. Cobb, L. Gumbrell, R.H. Begent, J.A. Hartley, D. Jodrell, Phase I study of sequence-selective minor groove DNA binding agent SJG-136 in patients with advanced solid tumors, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 15 (2009) 2140–2147.
[66] S. Kyle, H.D. Thomas, J. Mitchell, N.J. Curtin, Exploiting the Achilles heel of cancer: the therapeutic potential of poly(ADP-ribose) polymerase inhibitors in BRCA2-defective cancer, The British Journal of Radiology 81 (1) (2008) S6–11.
[67] S. Kummar, A. Chen, J. Ji, Y. Zhang, J.M. Reid, M. Ames, L. Jia, M. Weil, G. Speranza, A.J. Murgo, R. Kinders, L. Wang, R.E. Parchment, J. Carter, H. Stotler, L. Rubinstein, M. Hollingshead, G. Melillo, Y. Pommier, W. Bonner, J.E. Tomaszewski, J.H. Doroshow, Phase I study of PARP inhibitor ABT-888 in combination with topotecan in adults with refractory solid tumors and lymphomas, Cancer Research 71 (2011) 5626–5634.
[68] T.E. Fandy, Development of DNA methyltransferase inhibitors for the treatment of neoplastic diseases, Current Medicinal Chemistry 16 (2009) 2075–2085.
[69] A. Leonard, J. Rueff, G.B. Gerber, E.D. Leonard, Usefulness and limits of biological dosimetry based on cytogenetic methods, Radiation Protection Dosimetry 115 (2005) 448–454.
[70] R.A. Kleinerman, A.A. Romanyukha, D.A. Schauer, J.D. Tucker, Retrospective assessment of radiation exposure using biological dosimetry: chromosome painting, electron paramagnetic resonance and the glycophorin a mutation assay, Radiation Research 166 (2006) 287–302.
[71] M.M. Pinto, N.F. Santos, A. Amaral, Current status of biodosimetry based on standard cytogenetic methods, Radiation and Environmental Biophysics 49 (2010) 567–581.
[72] A. Sak, S. Grehl, P. Erichsen, M. Engelhard, A. Grannass, S. Levegrun, C. Pottgen, M. Groneberg, M. Stuschke, gamma-H2AX foci formation in peripheral blood lymphocytes of tumor patients after local radiotherapy to different sites of the body: dependence on the dose-distribution, irradiated site and time from start of treatment, International Journal of Radiation Biology 83 (2007) 639–652.
[73] C.E. Redon, A.J. Nakamura, K. Gouliaeva, A. Rahman, W.F. Blakely, W.M. Bonner, Qgamma-H2AX, an analysis method for partial-body radiation exposure using gamma-H2AX in non-human primate lymphocytes, Radiation Measurements 146 (2011) 877–881.
[74] T. Ohnishi, A. Takahashi, A. Nagamatsu, K. Omori, H. Suzuki, T. Shimazu, N. Ishioka, Detection of space radiation-induced double strand breaks as a track in cell nucleus, Biochemical and Biophysical Research Communications 390 (2009) 485–488.
[75] H. Zhou, M. Rodriguez, F. van den Haak, G. Nelson, R. Jogani, J. Xu, X. Zhu, Y. Xian, P.T. Tran, D.W. Felsher, P.J. Keall, E.E. Graves, Development of a micro-computed tomography-based image-guided conformal radiotherapy system for small animals, International Journal of Radiation Oncology, Biology, Physics 78 (2010) 297–305.
[76] C.E. Redon, J.S. Dickey, W.M. Bonner, O.A. Sedelnikova, gamma-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin, Advances in Space Research 43 (2009) 1171–1178.
[77] A. Andrievski, R.C. Wilkins, The response of gamma-H2AX in human lymphocytes and lymphocytes subsets measured in whole blood cultures, International Journal of Radiation Biology 85 (2009) 369–376.
[78] K. Rothkamm, S. Horn, gamma-H2AX as protein biomarker for radiation exposure, Annali dell'Istituto superiore di sanita 45 (2009) 265–271.
[79] E.C. Ford, P. Achanta, D. Purger, M. Armour, J. Reyes, J. Fong, L. Kleinberg, K. Redmond, J. Wong, M.H. Jang, H. Jun, H.J. Song, A. Quinones-Hinojosa, Localized CT-guided irradiation inhibits neurogenesis in specific regions of the adult mouse brain, Radiation Research 175 (2011) 774–783.
[80] M. Lobrich, N. Rief, M. Kuhne, M. Heckmann, J. Fleckenstein, C. Rube, M. Uder, In vivo formation and repair of DNA double-strand breaks after computed tomography examinations, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 8984–8989.
[81] K. Rothkamm, S. Balroop, J. Shekhdar, P. Fernie, V. Goh, Leukocyte DNA damage after multi-detector row CT: a quantitative biomarker of low-level radiation exposure, Radiology 242 (2007) 244–251.
[82] M.A. Kuefner, S. Grudzenski, S.A. Schwab, M. Wiederseiner, M. Heckmann, W. Bautz, M. Lobrich, M. Uder, DNA double-strand breaks and their repair in blood lymphocytes of patients undergoing angiographic procedures, Investigative Radiology 44 (2009) 440–446.
[83] M.A. Kuefner, S. Grudzenski, J. Hamann, S. Achenbach, M. Lell, K. Anders, S.A. Schwab, L. Haberle, M. Lobrich, M. Uder, Effect of CT scan protocols on x-ray-induced DNA double-strand breaks in blood lymphocytes of patients undergoing coronary CT angiography, European Radiology 20 (2010) 2917–2924.
[84] M.A. Kuefner, F.M. Hinkmann, S. Alibek, S. Azoulay, K. Anders, W.A. Kalender, S. Achenbach, S. Grudzenski, M. Lobrich, M. Uder, Reduction of X-ray induced DNA double-strand breaks in blood lymphocytes during coronary CT angiography using high-pitch spiral data acquisition with prospective ECG-triggering, Investigative Radiology 45 (2010) 182–187.

[85] F. Zwicker, B. Swartman, F. Sterzing, G. Major, K.J. Weber, P.E. Huber, C. Thieke, J. Debus, K. Herfarth, Biological in-vivo measurement of dose distribution in patients' lymphocytes by gamma-H2AX immunofluorescence staining: 3D conformal- vs. step-and-shoot IMRT of the prostate gland, Radiation Oncology 6 (2011) 62.
[86] R.F. Martin, W.A. Haseltine, Range of radiochemical damage to DNA with decay of iodine-125, Science 213 (1981) 896–898.
[87] O.A. Sedelnikova, E.P. Rogakou, I.G. Panyutin, W.M. Bonner, Quantitative detection of (125)IdU-induced DNA double-strand breaks with gamma-H2AX antibody, Radiation Research 158 (2002) 486–492.
[88] R.F. Martin, Induction of double-stranded breaks in DNA by binding with an 125i-labelled acridine, International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine 32 (1977) 491–497.
[89] A.I. Kassis, F. Fayad, B.M. Kinsey, K.S. Sastry, S.J. Adelstein, Radiotoxicity of an 125I-labeled DNA intercalator in mammalian cells, Radiation Research 118 (1989) 283–294.
[90] R.F. Martin, N. Holmes, Use of an 125I-labelled DNA ligand to probe DNA structure, Nature 302 (1983) 452–454.
[91] S.J. Adelstein, A.I. Kassis, Strand breaks in plasmid DNA following positional changes of Auger-electron-emitting radionuclides, Acta Oncologica 35 (1996) 797–801.
[92] I.G. Panyutin, R.D. Neumann, The potential for gene-targeted radiation therapy of cancers, Trends in Biotechnology 23 (2005) 492–496.
[93] O.A. Sedelnikova, I.V. Panyutin, R.D. Neumann, W.M. Bonner, I.G. Panyutin, Assessment of DNA damage produced by 125I-triplex-forming oligonucleotides in cells, International Journal of Radiation Biology 80 (2004) 927–931.
[94] I.V. Panyutin, O.A. Sedelnikova, W.M. Bonner, I.G. Panyutin, R.D. Neumann, DNA damage produced by 125I-triplex-forming oligonucleotides as a measure of their successful delivery into cell nuclei, Annals of the New York Academy of Sciences 1058 (2005) 140–150.
[95] M.P. Kelly, F.T. Lee, K. Tahtis, F.E. Smyth, M.W. Brechbiel, A.M. Scott, Radioimmunotherapy with alpha-particle emitting 213Bi-C-functionalized trans-cyclohexyl-diethylenetriaminepentaacetic acid-humanized 3S193 is enhanced by combination with paclitaxel chemotherapy, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 13 (2007) 5604s–5612s.
[96] M. Lassmann, H. Hanscheid, D. Gassen, J. Biko, V. Meineke, C. Reiners, H. Scherthan, In vivo formation of gamma-H2AX and 53BP1 DNA repair foci in blood cells after radioiodine therapy of differentiated thyroid cancer, Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine 51 (2010) 1318–1325.
[97] S.L. Tucker, F.B. Geara, L.J. Peters, W.A. Brock, How much could the radiotherapy dose be altered for individual patients based on a predictive assay of normal-tissue radiosensitivity?, Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology 38 (1996) 103–113
[98] C.N. Sprung, M. Chao, T. Leong, M.J. McKay, Chromosomal radiosensitivity in two cell lineages derived from clinically radiosensitive cancer patients, Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 11 (2005) 6352–6358.
[99] T. Leong, M. Borg, M. McKay, Clinical and cellular radiosensitivity in inherited human syndromes, Clinical Oncology (Royal College of Radiologist) 16 (2004) 206–209.
[100] I. Turesson, J. Nyman, E. Holmberg, A. Oden, Prognostic factors for acute and late skin reactions in radiotherapy patients, International Journal of Radiation Oncology, Biology, Physics 36 (1996) 1065–1075.
[101] M.H. Bourguignon, P.A. Gisone, M.R. Perez, S. Michelin, D. Dubner, M.D. Giorgio, E.D. Carosella, Genetic and epigenetic features in radiation sensitivity. Part II: implications for clinical practice and radiation protection, European Journal of Nuclear Medicine and Molecular Imaging 32 (2005) 351–368.
[102] M.H. Bourguignon, P.A. Gisone, M.R. Perez, S. Michelin, D. Dubner, M.D. Giorgio, E.D. Carosella, Genetic and epigenetic features in radiation sensitivity Part I: cell signalling in radiation response, European Journal of Nuclear Medicine and Molecular Imaging 32 (2005) 229–246.
[103] D.A. Chistiakov, V.A. Potapov, D.C. Khodirev, M.S. Shamkhalova, M.V. Shestakova, V.V. Nosikov, Genetic variations in the pancreatic ATP-sensitive potassium channel, beta-cell dysfunction, and susceptibility to type 2 diabetes, Acta Diabetologica 46 (2009) 43–49.
[104] J.M. Pollard, R.A. Gatti, Clinical radiation sensitivity with DNA repair disorders: an overview, International Journal of Radiation Oncology, Biology, Physics 74 (2009) 1323–1331.
[105] R.D. Wood, M. Mitchell, J. Sgouros, T. Lindahl, Human DNA repair genes, Science 291 (2001) 1284–1289.
[106] R.D. Wood, M. Mitchell, T. Lindahl, Human DNA repair genes, 2005, Mutation Research 577 (2005) 275–283.
[107] D. Twardella, J. Chang-Claude, Studies on radiosensitivity from an epidemiological point of view – overview of methods and results, Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology 62 (2002) 249–260.
[108] R. Huber, H. Braselmann, H. Geinitz, I. Jaehnert, A. Baumgartner, R. Thamm, M. Figel, M. Molls, H. Zitzelsberger, Chromosomal radiosensitivity and acute radiation side effects after radiotherapy in tumour patients – a follow-up study, Radiation Oncology 6 (2011) 32.
[109] F.B. Geara, L.J. Peters, K.K. Ang, J.L. Wike, S.S. Sivon, R. Guttenberger, D.L. Callender, E.P. Malaise, W.A. Brock, Intrinsic radiosensitivity of normal human fibroblasts and lymphocytes after high- and low-dose-rate irradiation, Cancer Research 52 (1992) 6348–6352.
[110] N.G. Burnet, J. Nyman, I. Turesson, R. Wurm, J.R. Yarnold, J.H. Peacock, Prediction of normal-tissue tolerance to radiotherapy from in-vitro cellular radiation sensitivity, Lancet 339 (1992) 1570–1571.
[111] A. Baeyens, H. Thierens, K. Vandenbulcke, L. De Ridder, A. Vral, The use of EBV-transformed cell lines of breast cancer patients to measure chromosomal radiosensitivity, Mutagenesis 19 (2004) 285–290.
[112] A. Baeyens, K. Vandenbulcke, J. Philippe, H. Thierens, L. De Ridder, A. Vral, The use of IL-2 cultures to measure chromosomal radiosensitivity in breast cancer patients, Mutagenesis 19 (2004) 493–498.
[113] F.B. Geara, L.J. Peters, K.K. Ang, J.L. Wike, W.A. Brock, Radiosensitivity measurement of keratinocytes and fibroblasts from radiotherapy patients, International Journal of Radiation Oncology, Biology, Physics 24 (1992) 287–293.
[114] R.S. Vasireddy, C.N. Sprung, N.L. Cempaka, M. Chao, M.J. McKay, H2AX phosphorylation screen of cells from radiosensitive cancer patients reveals a novel DNA double-strand break repair cellular phenotype, British Journal of Cancer 102 (2010) 1511–1518.
[115] S. Golfier, G. Jost, H. Pietsch, P. Lengsfeld, F. Eckardt-Schupp, E. Schmid, M. Voth, Dicentric chromosomes and gamma-H2AX foci formation in lymphocytes of human blood samples exposed to a CT scanner: a direct comparison of dose response relationships, Radiation Protection Dosimetry 134 (2009) 55–61.
[116] A. Menegakis, A. Yaromina, W. Eicheler, A. Dorfler, B. Beuthien-Baumann, H.D. Thames, M. Baumann, M. Krause, Prediction of clonogenic cell survival curves based on the number of residual DNA double strand breaks measured by gammaH2AX staining, International Journal of Radiation Biology 85 (2009) 1032–1041.
[117] C.E. Rube, A. Fricke, R. Schneider, K. Simon, M. Kuhne, J. Fleckenstein, S. Graber, N. Graf, C. Rube, DNA repair alterations in children with pediatric malignancies: novel opportunities to identify patients at risk for high-grade toxicities, International Journal of Radiation Oncology, Biology, Physics 78 (2010) 359–369.
[118] A.A. Goodarzi, P.A. Jeggo, Irradiation induced foci (IRIF) as a biomarker for radiosensitivity, Mutation Research (2011).
[119] P. Porcedda, V. Turinetto, E. Lantelme, E. Fontanella, K. Chrzanowska, R. Ragona, M. De Marchi, D. Delia, C. Giachino, Impaired elimination of DNA double-strand break-containing lymphocytes in ataxia telangiectasia and Nijmegen breakage syndrome, DNA Repair 5 (2006) 904–913.
[120] F. Abbaszadeh, P.H. Clingen, C.F. Arlett, P.N. Plowman, E.C. Bourton, M. Themis, E.M. Makarov, R.F. Newbold, M.H. Green, C.N. Parris, A novel splice variant of the DNA-PKcs gene is associated with clinical and cellular radiosensitivity in a patient with xeroderma pigmentosum, Journal of Medical Genetics 47 (2010) 176–181.
[121] A. Leskovac, D. Vujic, M. Guc-Scekic, S. Petrovic, I. Joksic, P. Slijepcevic, G. Joksic, Fanconi anemia is characterized by delayed repair kinetics of DNA double-strand breaks, The Tohoku Journal of Experimental Medicine 221 (2010) 69–76.
[122] T.A. Kato, P.F. Wilson, H. Nagasawa, M.M. Fitzek, M.M. Weil, J.B. Little, J.S. Bedford, A defect in DNA double strand break processing in cells from unaffected parents of retinoblastoma patients and other apparently normal humans, DNA Repair 6 (2007) 818–829.
[123] E.C. Bourton, P.N. Plowman, D. Smith, C.F. Arlett, C.N. Parris, Prolonged expression of the gamma-H2AX DNA repair biomarker correlates with excess acute and chronic toxicity from radiotherapy treatment, International Journal of Cancer. Journal International du Cancer (2011).
[124] T.A. Kato, P.F. Wilson, H. Nagasaw, Y. Peng, M.M. Weil, J.B. Little, J.S. Bedford, Variations in radiosensitivity among individuals: a potential impact on risk assessment?, Health Physics 97 (2009) 470–480
[125] J. Werbrouck, K. De Ruyck, L. Beels, A. Vral, M. Van Eijkeren, W. De Neve, H. Thierens, Prediction of late normal tissue complications in RT treated gynaecological cancer patients: potential of the gamma-H2AX foci assay and association with chromosomal radiosensitivity, Oncology Reports 23 (2010) 571–578.
[126] J. Werbrouck, F. Duprez, W. De Neve, H. Thierens, Lack of a correlation between gammaH2AX foci kinetics in lymphocytes and the severity of acute normal tissue reactions during IMRT treatment for head and neck cancer, International Journal of Radiation Biology 87 (2011) 46–56.
[127] J. Fleckenstein, M. Kuhne, K. Seegmuller, S. Derschang, P. Melchior, S. Graber, A. Fricke, C.E. Rube, C. Rube, The impact of individual in vivo repair of DNA double-strand breaks on oral mucositis in adjuvant radiotherapy of head-and-neck cancer, International Journal of Radiation Oncology, Biology, Physics (2010).
[128] M. Lobrich, A. Shibata, A. Beucher, A. Fisher, M. Ensminger, A.A. Goodarzi, O. Barton, P.A. Jeggo, gammaH2AX foci analysis for monitoring DNA double-strand break repair: strengths, limitations and optimization, Cell Cycle 9 (2010) 662–669.
[129] S.M. Bentzen, Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology, Nature Reviews Cancer 6 (2006) 702–713.
[130] M.V. Sokolov, J.S. Dickey, W.M. Bonner, O.A. Sedelnikova, gamma-H2AX in bystander cells: not just a radiation-triggered event, a cellular response to stress mediated by intercellular communication, Cell Cycle 6 (2007) 2210–2212.
[131] J. Bartkova, Z. Horejsi, K. Koed, A. Kramer, F. Tort, K. Zieger, P. Guldberg, M. Sehested, J.M. Nesland, C. Lukas, T. Orntoft, J. Lukas, J. Bartek, DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis, Nature 434 (2005) 864–870.

[132] K.M. Prise, M. Folkard, V. Kuosaite, L. Tartier, N. Zyuzikov, C. Shao, What role for DNA damage and repair in the bystander response?, Mutation Research 597 (2006) 1–4
[133] O.A. Sedelnikova, W.M. Bonner, GammaH2AX in cancer cells: a potential biomarker for cancer diagnostics, prediction and recurrence, Cell Cycle 5 (2006) 2909–2913.
[134] C.E. Redon, J.S. Dickey, A.J. Nakamura, I.G. Kareva, D. Naf, S. Nowsheen, T.B. Kryston, W.M. Bonner, A.G. Georgakilas, O.A. Sedelnikova, Tumors induce complex DNA damage in distant proliferative tissues in vivo, Proceedings of the National Academy of Sciences of the United States of America 107 (2010) 17992–17997.
[135] T.Z. Belhocine, F.G. Blankenberg, The imaging of apoptosis with the radiolabelled annexin A5: a new tool in translational research, Current Clinical Pharmacology 1 (2006) 129–137.
[136] F.G. Blankenberg, J.L. Vanderheyden, H.W. Strauss, J.F. Tait, Radiolabeling of HYNIC-annexin V with technetium-99m for in vivo imaging of apoptosis, Nature Protocols 1 (2006) 108–110.
[137] J.F. Tait, C. Smith, Z. Levashova, B. Patel, F.G. Blankenberg, J.L. Vanderheyden, Improved detection of cell death in vivo with annexin V radiolabeled by site-specific methods, Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine 47 (2006) 1546–1553.
[138] L.E. Edgington, A.B. Berger, G. Blum, V.E. Albrow, M.G. Paulick, N. Lineberry, M. Bogyo, Noninvasive optical imaging of apoptosis by caspase-targeted activity-based probes, Nature Medicine 15 (2009) 967–973.
[139] R. Aloya, A. Shirvan, H. Grimberg, A. Reshef, G. Levin, D. Kidron, A. Cohen, I. Ziv, Molecular imaging of cell death in vivo by a novel small molecule probe, Apoptosis: An International Journal on Programmed Cell Death 11 (2006) 2089–2101.
[140] B. Cornelissen, V. Kersemans, S. Darbar, J. Thompson, K. Shah, K. Sleeth, M.A. Hill, K.A. Vallis, Imaging DNA damage in vivo using {gamma}H2AX-targeted immunoconjugates, Cancer Research 71 (2011) 4539–4549.
[141] W. Li, F. Li, Q. Huang, J. Shen, F. Wolf, Y. He, X. Liu, Y.A. Hu, J.S. Bedford, C.Y. Li, Quantitative, noninvasive imaging of radiation-induced DNA double-strand breaks in vivo, Cancer Research 71 (2011) 4130–4137.