Advances in biological dosimetry

A Ivashkevich[1,2], T Ohnesorg[3], C E Sparbier[1], H Elsaleh[1,4]
[1]Radiation Oncology, Canberra Hospital, Garran, ACT, 2605, Australia
[2]Australian National University, Canberra ACT 2600, Australia
[3]Molecular Development, Murdoch Children's Research Institute, Melbourne, VIC, 3052, Australia
[4]College of Medicine Biology and Environment, Australian National University, Canberra ACT 2600, Australia

E-mail: alesia.ivashkevich@act.gov.au

Abstract. Rapid retrospective biodosimetry methods are essential for the fast triage of persons occupationally or accidentally exposed to ionizing radiation. Identification and detection of a radiation specific molecular 'footprint' should provide a sensitive and reliable measurement of radiation exposure. Here we discuss conventional (cytogenetic) methods of detection and assessment of radiation exposure in comparison to emerging approaches such as gene expression signatures and DNA damage markers. Furthermore, we provide an overview of technical and logistic details such as type of sample required, time for sample preparation and analysis, ease of use and potential for a high throughput analysis.

1. Introduction

As one of the most recent nuclear accidents, the Fukushima Daiichi nuclear disaster in 2011, clearly demonstrated, efficient contingency plans are required to assist with population triage in case of large scale radiation exposure. Biological dosimetry complements physical measurements and in some circumstances might be the only available approach to provide a reliable dose estimate. It is performed in combination with clinical assessment during the early response phase of a radiation emergency in order to retrospectively assess the imparted radiation dose and assist to determine the potential biological effects [1]. Biodosimetry methods are of great assistance for the evaluation of biological effects of the imparted radiation dose, although some caution is required for interpretation of the results. Increased individual susceptibility to radiation effects might result in a dose overestimation. Despite the well demonstrated usefulness of cytogenetic biodosimetry, the practical applications are limited by the time required to run the assay. Therefore, extensive developments and validation of novel assays were undertaken during the recent two decades to improve biodosimetry.

2. Current approaches, limitations and new developments in biodosimetry

2.1. Biomarkers in biodosimetry

The definition of a biomarker is 'any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological' [2]. Radiation biomarkers can be used for: 1) estimation or validation of received doses, 2) investigation of individual susceptibility, 3) early detection of radiation-induced health effects.

Conventional cytogenetic biodosimetry assays use chromosomal aberrations as indication of exposure, and scoring of dicentrics is considered the 'gold standard' of biodosimetry. Measured cytogenetic changes are the end-product of processing and cellular responses to inflicted damage. In contrast, emerging approaches, such as detection of DNA damage and gene expression changes, are indicative of initial cellular processes occurring shortly after exposure (Figure 1).

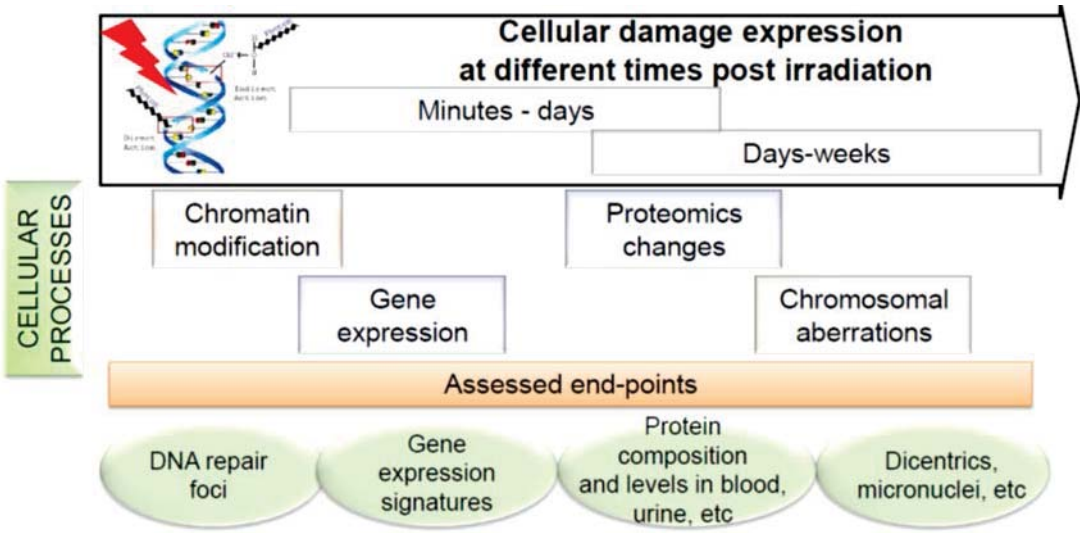

Figure 1. Overview of novel and existing biodosimetry methods.

Depending on the detected event in the sequence of DNA damage elicited molecular responses, current markers of radiation exposure can be broadly classified into:

- biomarkers detecting direct damage to DNA
- epigenetic modifications
- changes to gene expression (transcriptional) and altered protein composition and levels (translational changes)
- induced somatic and germline mutations/variants
- chromosomal changes as detected by cytogenetic biomarkers

2.2. Ideal biomarker

For a biomarker to be utilized, the assay accuracy of the dose estimate is of paramount importance to aid a reliable retrospective estimation of radiation doses in exposed individuals. Apart from being rapid, specific, sensitive, reproducible, an ideal bioassay should be able to distinguish the nature of exposure, and provide a reliable dose estimate irrespective of the exposure time. Biodosimetry assays also have to be high throughput and cost-effective.

It is also required to accurately measure and potentially differentiate between low and high dose, acute or prolonged, and external or internal exposure (Figure 2).

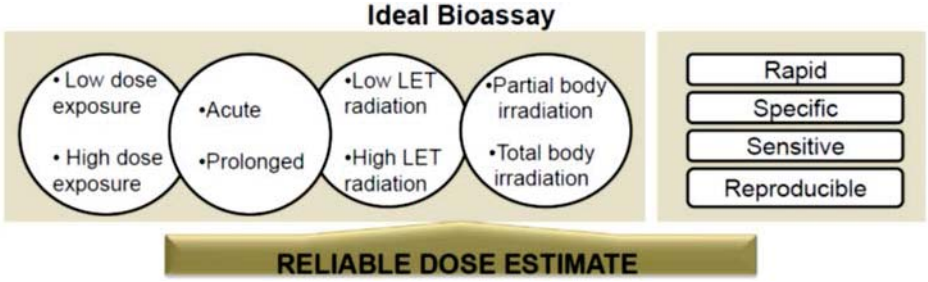

Figure 2. Essential features of an 'ideal' assay for optimal biodosimetry.

With a wide choice of methods, appropriate assays can be utilized depending on the situation. E.g. in emergency settings, for clinical management of accidental exposure, the expected dose assessment has to be accurate enough to predict clinical symptoms in order to appropriately treat the post accidental syndrome. Biological methods for detection of radiation found further applications in clinical settings for assessment of tumour and normal tissue sensitivity and response of tumours to chemoradiation [3-6].

2.3. Sample access and processing time

Biological material accessible for biodosimetry includes blood, urine, buccal cells, skin, hair follicles, etc. Peripheral blood lymphocytes (PBL) represent the most suitable sample as cells travel around the entire body; therefore, measurements performed in PBL enable the assessment of partial body exposure. About 2% of PBL are located in blood, continuously exchanging with those located in tissues and organs [6]. PBL can remain in circulation for several years allowing measurements for an extended time post exposure. PBL represent a suitable biological sample for all bioassays. However conventional cytogenetics takes longer than many other bioassays as the protocol requires mitogen (phytohemaglutinin) stimulation to activate proliferation of normally G0 lymphocytes. Cells have to undergo mitosis in order to exhibit damage detectable as translocations/micronuclei. Analysis of DNA damage repair foci (γH2AX) or gene expression changes is usually performed in PBL, too. These assays require no stimulation and the dose assessment can be performed within 12 hrs post exposure.

2.4. Dose response

Accurate dose quantification can only be performed using calibration dose response curves. Radiation dose dependent reproducible changes in marker expression, detectable with the selected assay are a prerequisite for successful biodosimetry methodology. Reduced sensitivity towards lower range doses, saturation of the response, and the detection threshold are potential limitations to consider. Saturation of the measured response limits method's ability to detect high doses and affect the time course of the response decay.

Dose response relationship for the yield of dicentrics produced by photon sources is fitted with a linear-quadratic model, which is consistent with single- and two-track hit models of aberration formation, $y = \alpha D + \beta D^2$ [7]. If exposure is spread over a certain period of time, damage repair will occur, and the quadratic component of the dose response relationship will gradually disappear. In chromosomal aberrations assay, the shape of the dose response curve depends strongly on Relative Biological Efficiency (RBE). Fission neutrons induced a significantly higher yield of dicentrics than that caused by low-Linear Energy Transfer (LET) sources. The linear component of the model, corresponding to damage caused by single-tracks, is predominant with fission neutrons. γH2AX foci show a linear dose response across a broad dose range [8, 9] and the assay is sensitive to doses as low as a few milligrays. In gene expression based biodosimetry, a linear dose-response relationship has been obtained for some genes over a dose range of 1–3 Gy [10], 0.5–4 Gy [11] and down to doses as low as 2 cGy [12].

2.5. In vivo validation of dose response

Radiation dose-response relationships are frequently analysed *in vitro* and have to be validated *in vivo*. Patients undergoing thyroid cancer treatment and total body irradiation during the treatment course for haematological malignancies [13] are main cohorts which were used to validate *in vitro* established assays. Although generally being in a good agreement with a physical dose, results of biological dosimetry tend to overestimate the actual physical dose [13]. Biodosimetry data relying on cancer patients have to be interpreted with caution, as it might not entirely reflect the situation in healthy cohorts due to the higher chance of patients carrying radiation susceptibility/resistance, and a potential exposure to previous chemo-radiation.

2.6. Discrimination between partial versus total body exposure

In case of chromosomal aberrations and γH2AX foci, homogeneous exposure yields a Poisson distribution of the aberrations and DNA repair foci, whereas inhomogeneous exposure results in contaminated Poisson distribution [14, 15]. Gene expression based biodosimetry approach lacks the capacity to distinguish this kind of difference in exposure.

2.7. High throughput approaches

The dicentric assay requires two days from sampling to results and it has limited sample processing capacity for mass screening following a nuclear incident. Standard biological dosimetry procedure (500 manually scored metaphases) is suitable for a few dose estimations and it cannot be performed in a timely manner in the case of a large-scale accident. A recent estimation, which included the 15 European Union countries where biological dosimetry is established, gave a total capacity for dosimetric triage of about 1500 cases per week [16]. Automated detection of dicentrics was introduced to enable a high throughput analysis. It has greatly reduced the time needed for dose estimation in whole- and partial-body accidental exposures without compromising its accuracy [7, 17]. Image analysis automation included development of a specialised software and algorithms for a high throughput counting of DNA repair foci [4].

2.8. Optimisation of gene expression biodosimetry for high-throughput applications

Detection of changes in gene expression has shown to be a promising biodosimetry approach [10, 18]. As a differential induction of alternative transcription in some genes (*MDM2, CDKN1a*) by irradiation is well documented [19, 20], one of the most recent studies suggested utilizing parts of the genes not induced by irradiation as intragenic control in order to decrease intra-individual variability in gene expression analysis [21].

Combination of automated biodosimetry assays is incorporated in so-called RABiT-infrastructure created to support ultra high throughput biodosimetry [22, 23].

3. Conclusions and Outlook

As each assay possesses certain limitations (scoring dicentrics is difficult to apply for triage of a large scale accident, the γH2AX assay is useful only within a short time window post exposure) and unique advantages (both γH2AX and gene expression analyses are sensitive and rapid bioassays), a combination of methods depending on the specific situation of a radiation accident will most likely allow the most accurate retrospective dose evaluation.

Despite recent advances and spurring developments of new assays, further improvement and validation will result in better reliability and accuracy of biodosimetry. Establishment of global infrastructure integrating new developments and access to required resources will assure better preparedness at all levels for every kind of nuclear disasters.

4. References

[1] International Atomic Energy Agency 1998 Diagnosis and treatment of radiation injuries *Safety Reports* **2** (Vienna, Austria).
[2] World Health Organisation 1993 Biomarkers and risk assessment: concepts and principles *EHC 155* (Geneva, Switzerland).
[3] Minicucci E M *et al* 2014 *World J. Clin. Oncol.* **5** 93-102
[4] Ivashkevich A N *et al* 2011 *Mutat. Res.* **711** 49-60
[5] Ivashkevich A *et al* 2012 *Cancer Lett.* **327** 123-33
[6] International Atomic Energy Agency 2011 Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies (Vienna, Austria)
[7] Prasanna P G S *et al* 2002 AFRRI's Gamma-Ray, X-Ray, and Fission-Neutron Calibration Curves for the Lymphocyte Dicentric Assay: Application of a Metaphase Finder System. *Armed Forces Radiobiology Research Institute* (Bethesda, Maryland)
[8] Beels L *et al* 2010 *Int. J. Radiat. Biol.* **86** 760-8
[9] Moroni M *et al* 2013 *Int. J. Mol. Sci.* **14** 14119-35
[10] Grace M B *et al* 2002 *Int. J. Radiat. Biol.* **78** 1011-21
[11] Kang C M *et al* 2003 *Radiat. Res.* **159** 312-9
[12] Amundson S A, Fornace A J, Jr. 2003 *Health Phys.* **85** 36-42
[13] Dossou J *et al* 2000 *Int. J. Radiat. Oncol. Biol. Phys.* **46** 123-9
[14] Hilali A *et al* 1991 *Radiat. Res.* **128** 108-11
[15] Redon C E *et al* 2010 *PLoS One* **5** e15544
[16] Wojcik A *et al* 2010 *Radiat. Prot. Dosimetry* **138** 397-401
[17] Vaurijoux A *et al* 2012 *Radiat. Res.* **178** 357-64
[18] Kabacik S *et al* 2011 *Int. J. Radiat. Biol.* **87** 115-29
[19] Sprung C N *et al* 2011 *PLoS One* **6** e25758
[20] Forrester H B *et al* 2012 *PLoS One* **7** e53358
[21] Forrester H B *et al* 2014 *Radiat. Res.* **181** 314-23
[22] Garty G *et al* 2011 *Int. J. Radiat. Biol.* **87** 754-65
[23] Turner H C *et al* 2014 *Radiat. Environ. Biophys.* **53** 265-72