Defence Life Science Journal, Vol. 2, No. 3, July 2017, pp. 270-277, DOI : 10.14429/dlsj.2.11664

REVIEW PAPER

Radiation Biomarkers : Applications in Triage Management of Radiation Victims

Saurabh Mishra, and Raj Kumar*

Institute of Nuclear Medicine and Allied Sciences, Delhi - 110 054, India
**E-mail: rajkumar790@yahoo.com*

ABSTRACT

Human exposure to ionising radiation disrupts normal metabolic processes in cells and organs by inducing complex biological responses that interfere with gene and protein expression. Conventional dosimetry, monitoring of prodromal symptoms and peripheral lymphocyte counts are of limited value as organ- and tissue-specific biomarkers for personnel exposed to radiation, particularly, weeks or months after exposure. Analysis of metabolites generated in known stress-responsive pathways by molecular profiling helps to predict the physiological status of an individual in response to environmental or genetic perturbations. There is a need for research to rapidly determine an individual's absorbed dose and its potential health effects after a potential radiological or nuclear event that could expose large portions of a population to ionising radiation. Studies on biomarker identification after radiation exposure could contribute in biodosimetry, identifying individual dose absorbed, as well as biologic response, and administering immediate and proper medical care. In the recent scenario development of biomarker is major thrust area. Articles related to gene biomarker, protein biomarker and metabolic biomarker are reviewed in order to sketch an overview on the recent advances related to developing an biomarker to assess the radiation induced toxicity.

Keywords: Ionising radiation; Gene biomarker; Protein biomarker; Radiation dosage

1. INTRODUCTION

Biomarkers are biological scale that can be measured and evaluated as an indicator of pathologic processes that indicates pharmacological response upon therapeutic Intervention. Radiation biomarkers are specifically indicator of radiation doses absorbed by a radiation victim. Radiation biomarkers are also help to suggest the necessity of medical interventions such as countermeasure applications. Since, biomarkers are quantifiable in nature; they can be used to characterise indirect or direct drug performance, dose selection and potential safety issues related to candidate drug administration[1].

In general biomarkers are categorised as

(a) Diagnostic biomarkers categorises a person by the presence or absence of a particular disease or physiological or pathophysiological state
(b) Prognostic biomarker are those which categorises patients by level of risk for disease progression or occurrence and
(c) A predictive biomarker categorises patients by their likelihood of response to treatment with respect to no treatment. It can predict a favourable or adverse effect of a particular treatment[2].

Biological markers for human population for ionising radiation (IR) exposure are of great interest for analysing tissue injury in biodosimetry during nuclear incidents or accidents. The current approach for radiation biodosimetry include physical effects, such as time of action, kinetics of blood lymphocyte and assessment of cytogenetic damage in blood lymphocytes[3,4]. Limitations of these methods are they are time-consuming and thus not suitable for triage exposed persons during radiation eventuality.

Received : 08 May 2017, Revised : 10 June 2017
Accepted : 15 June 2017, Online published : 02 August 2017

2. GENOMIC BIOMARKER OF RADIATION DOSIMETRY

Genomic analysis provides excellent tools to identify genes involved in DNA damage response and repair functions. DNA is sensitive towards radiation and serves as a critical cellular target and the potential of the cell to repair DNA damage determines its fate post exposure. Radiation induced damage in DNA damage by different means including DNA-protein cross-links modulation, DNA Single strand breaks (SSBs), alteration in sugar and nitrogen backbone, bulky lesions formation i.e. clusters of base and sugar damage and double strand breaks (DSBs)[5]. The immediate effect of radiation induced DNA damage is activation of the DNA repair mechanism and the activation of cell cycle checkpoints, induction of down-stream signalling for cellular responses such as apoptosis that removes damaged cells. The main repair mechanism is base excision repair (BER) that is responsible for the elimination of damaged bases and single-strand breaks in DNA by involvement of DNA polymerase and ligation of DNA ends[6]. Another major pathway to repair bulky DNA damages is Nucleotide excision repair (NER) that repairs helical distortion[7]. The proteins of NER are involved in repairing oxidative damage through activation of BER that includes

XPC (Xerodermapigmentosum, complementation group C) and XPG (Xerodermapigmentosum, complementation group G)[8]. There are several genes of NER which are up-regulated by radiation, including XPC and Damage Specific DNA binding protein 2 (DDB2)[9,10]. Ionising radiation is known to activate transcript and several components of cell cycle regulators i.e. CDKN1A (p21), Cyclin G1 (CCNG1), GADD45a, CHK2-thr68 and apoptosis regulating genes i.e. (BAX and BBC3[11-13]. However, there is very less information regarding co-exposure of confounding factors that can affect the utility of individual biomarker of radiation biodosimetry[14]. The *ex vivo* model of human blood irradiation has been used to investigate the early radiation induced biological responses for potential biodosimetry applications[4]. There is a study that reports about the transcriptional response of well-known DNA repair, cell cycle and apoptosis regulating genes after irradiation

With the advancement of high end DNA microarrays technique and next-generation Sequencing (NGS), screening and identification of radiation sensitive genes are possible. Recently there are several studies that have demonstrated the success and functional methodology involve in assessment of doses dependent gene expression levels in cellular models[14-16]. To analyse human dosimetry to determine the dose rate effect on the gene expression study was conducted using whole blood samples from individual which are exposed to acute radiation dose rate with low radiation dosages[9]. Among the sets of genes responded to low dose rate (LDR 0.56 Gy) were XPC, DDB2, POLH, GADD45A and PCNA. The findings suggested that the gene expression response in the cells exposed to LDR (0.56 Gy) continuously to 24 h was different as compared to the cells which were exposed with acute exposure at 4.45 Gy. In the same study another 143 genes were identified that were sensitive towards radiation. Gene ontology analysis of these genes was revealed that there is enrichment of two processes: glycolysis and monosaccharide metabolic process. Genes involved in these two categories were the members of the glycolysis pathway (lactatedehydrogenase A, glyceraldehyde 3-phosphatedehydrogenase, 6-phospho-fructokinase type C, enolase 1, and hexokinase 2. These genes were expressed at lower levels in the cells that exposed to protracted dose[17]. Another study that identified more than 20 genes that indicated gene expression response not much affected with the dose rate. There were up regulated i.e. AEN and CDKN1A and down regulated i.e. MYC and E2F5 gene in both LDR and acute exposure cases[18].

There is another pattern of gene expression reported in which gene expression induced only in low dose radiation but not in acute exposure. There were two types of genes in this group, one in which the genes were up regulated by LDR only, but not with acute doses (RBM3 and GRM2) and in another case, where genes were down regulated by LDR only but not with acute doses (DUSP3 and ID2, Fig. 3)[19]. This preliminary assessment of differential gene expression with low dose rate radiation exposure suggested that there are genes that can be distinguish based on different dose-rate strength of applied radiation[20]. Interestingly, there are some genes such as APOBEC3H, FDXR and PHLDA3 that can induce at all doses by low dose rate and acute radiation exposure[17]. However, the change in gene expression beyond 4.45 Gy dose was higher in the acute exposure group as compared to LDR exposed groups of cells.

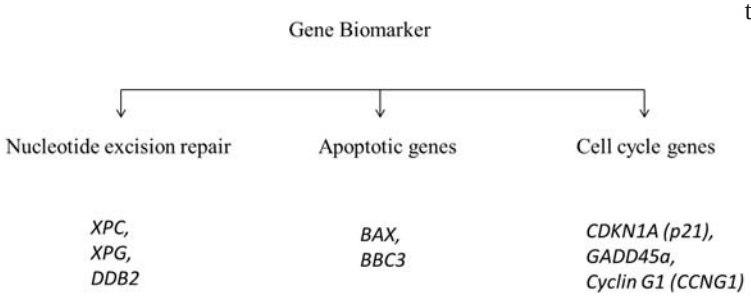

Figure.1 Radiation induced gene biomarker. Up regulated nucleotide excision repair genes (XPC, XPG, DDB2), Apoptotic genes (BAX, BBC3) and cell cycle genes (CDKN1, GADD45α, Cyclin G1).

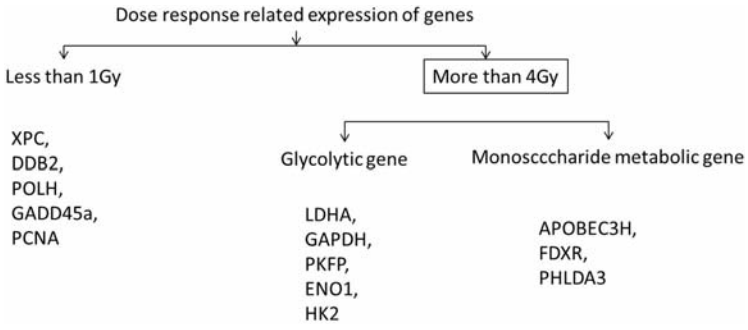

Figure 2. Radiation dose response related expression of genes. Genes responding to 1 Gy and genes responding on more than 5 Gy exposure.

3. PROTEIN BIOMARKER FOR RADIATION EXPOSURE

3.1 γ-H2AX

Radiation induced DNA double-strand break (DSB) is a cytotoxic form of DNA alteration. If this damage is not repaired correctly it can leads to genomic instability, chromosome aberrations and mutations[21,22]. A linear correlation was established in double strand breaks (DSBs) and radiation dose. It has been reported that 1.0Gy of radiation induces approximately 20-40 DSBs per cell nucleus[23]. H2AX play a significant role in DNA damage repair. Phosphorylation of H2AX is mediated by the phosphoinositide-3-kinase-related kinase (PIKK) family members ATM and DNA-PK following ionising irradiation[24]. Whereas, ATR and DNA-PK appear to be involved in γ-H2AX formation at the sites of replication-associated breaks[25]. The measurement of γ-H2AX foci is done following low radiation exposure as 1 mGy, and foci yields have been shown to increase linearly with increasing radiation doses[22]. Scoring of foci is the most sensitive method for γ-H2AX analysis. A single DSB results in the phosphorylation of thousands of H2AX proteins molecules over chromatin domain of several mega bases of DNA either side of the break.

3.2 Amylase, Flt3-ligand and C-reactive Protein as Bio-indicator of Radiation Damage

Reduction in amylase level is considered as a direct indicator of radiation-induced damage of the parotid gland. While Flt3-ligand, a hematopoietic cytokine, is considered as bio-indicator of bone marrow damage[26]. Both of these parameters are relatively easy to analyse quantitatively in serum/plasma using a clinical blood chemistry analyser or using ELISA[27]. The variation in the level of C-reactive protein level, (secreted component by hepatocytes cells during inflammation), presents an accurate measurement of the degree of systemic inflammation. It is not only specific to radiation exposure but it can be modulated in case of cancer, rheumatoid arthritis and cardiovascular diseases[28].

3.3 Cytokines, Chemokines, and other Proteins

In the recent time cytokines and chemokines are considered a sensitive biomarker of radiation exposure. G-CSF has been reported to be up-regulated in irradiated mouse and non-human primates (NHPs) and plays an important role in ameliorating radiation injury[29-32]. Additionally, several cytokines, chemokines and other proteins have been identified as candidate biomarkers of radiation injury over the last few years, as tested in total-body and partial-body exposure situation in murine and NHP models[33-36]. Among all, interleukin-6 (IL-6), C-reactive protein (CRP), serum amyloid A (SAA), growth arrest and DNA damage-inducible 45 (GADD45) proteins, FMS-like tyrosine kinase 3 ligand (flt3L) and salivary α- amylase are some prominent members of radiation sensitive proteins biomarkers. Previously, interleukin-18 (IL-18) has been reported up-regulated at 5–10 Gy ^{60}Co γ-irradiation. IL-18 serum concentrations are also correlated with radiation dose in mini-pigs, NHPs, and mice [37].

3.4 Peripheral Blood Counts

There are several studies that use peripheral blood cell counts as radiation biomarker of post irradiation situation. The cells analysed are granulocytes, lymphocytes, leukocytes, and platelets. Peripheral blood cell counts test has significance as it correlate radiation dose exposure not restricted at early time points (1 day or 2 days) but can be extended to the late phase (up to 4 weeks) after radiation exposure[38]. Peripheral blood cell counts have been used to monitor the health of the victims of radiation related accidents, support the use of this parameter as a diagnostic tool[39].

4. CYTOGENETIC BIOMARKERS

4.1 Dicentrics

Dicentric chromosomes (i.e. chromosomes with two centromeres) are induced by ionising radiation. They are substantially stable within non-dividing cells such as lymphocytes. The stability (half-life) of blood lymphocytes in order of months/years depends upon the sub-population[40], thus the dicentric exists as biomarker of choice for radiation exposure. Using dicentric assay, individual dose assessment can be established in the victims exposed with whole-body radiation exposure as low as 100 mGy. Since, the dicentric assay is very tedious, expertise oriented and time consuming, it may not the choice of diagnosis during mass radiation exposure to general public. Continuous efforts have been made to develop an automated system to provide reproducible results within short period of time[41]. In continuation to acute whole-body exposures the dose estimation for partial-body and protracted exposure can be achieved by scoring dicentrics in lymphocytes.

4.2 Premature Chromosome Condensation

Premature chromosome condensation (PCC) is technique can be used to condense the chromosomes in quiescent and[42]. In process to cycling cells, it is possible to score ring chromosomes, dicentrics and translocations, if the PCC assay and FISH is combined together with chromosome painting or c-banding[43,44]. PCC has been considered as the most useful technique for assessing high dose acute exposures of low LET radiation[45,46]. PCC technique is successfully utilised in assessing some cases of exposure, especially for the problems of the radioprotection and assessment of received doses after medical imaging (X-ray and nuclear medicine)[47].

4.3 Telomere Length

Telomeres are heterochromatic region composed of DNA repeats (TTAGGG) that binds to an array of specialised proteins which is situated at the end of chromosomes. Length of the repeats of telomere and the integrity of telomere binding proteins (TBP), these both are important for telomere protection[48]. Radiation exposure was known to be impaired telomere function that can lead to genomic instability, cancer progression, genetic instability, increased radiation sensitivity, loss of cellular viability and senescence[49]. It is has been reported earlier that short telomeres participates in inducing genomic instability in the aged progeny of irradiated cells[50]. The telomere length modulates chromosome *in vitro* radiosensitivity in healthy individuals[51]. The analysis of the telomere lengths in the study of persons exposed to radiation shows potential to exist as a radiation biomarker[52].

4.4 Micronuclei

Micronuclei are formed when the intact chromosomes or fragments are not properly separated to daughter nuclei during anaphase rather it remains in the cytoplasm after cell division. It could be visualised as a small spherical objects using conventional DNA dye[53,54]. Such analysis could be made on fresh blood or frozen peripheral blood lymphocytes[55]. Micronuclei analysis by cytokinesis-blocked micronucleus (CBMN) assay exhibits a great potential as a biomarker for individual's radiosensitivity[56].

4.5 Metabolic Biomarker

Radiation metabolomics is considered as the chemical fingerprinting of biological fluids to identify latent, endogenous small molecules having altered concentrations in a dose-dependent manner following radiation exposure[57]. Responding to the potential threat of nuclear and radiological terrorism Centre for High-Throughput Minimally Invasive Radiation Biodosimetry (CMCR) was established to develop biodosimeters which are field-deployable.

A study performed on the mice which were irradiated and urine samples were analysed on ^{1}H NMR (proton NMR). Profile of urine samples revealed the presence of lactate, alanine, glutamine, glutamate and alanine some other metabolites like hippurate, taurine, trimethyl amine (TMA), a-ketoglutarate (α-KG) and 2-ketoisocaproate were also present (Fig. 3)[58]. NMR profiling of serum of irradiated mice was characterised by the predominance of lipids along with creatine, amino acids and organic acids[59]. Comparative analysis revealed about the significant changes were observed mainly in metabolites associated with energy metabolism (succinate, a-KG, citrate), gut flora metabolites [hippurate, TMA], osmolytes (betaine, sarcosine), amino acids (branched chain amino acids, isoleucine, phenylalanine, taurine) and creatinine in urine samples (Fig. 3) of irradiated mice[60]. Further, alteration in different metabolites at different phases of radiation sickness were found and reported, dramatic change in metabolic profile was reported at post irradiation time with significant increase in almost all metabolites[61]. On the other hand, metabolic changes observed in the serum were branched chain amino acid, lipids and lactate[62]. Some of the membrane metabolites viz., choline and phosphoethanolamine were increased only during post irradiation[63]. Most of the metabolites in the serum and urine of irradiated animals were found to be increased after sub lethal dose of whole body radiation. Most of the changes observed in urine were Kreb's cycle intermediates along with other energy metabolites[64]. After radiation exposure, the bioenergetic status of the body might have resulted from increased energy demands and were, therefore, suggestive of changes in energy metabolism (Fig. 3) after radiation exposure[53]. Interestingly, longer chain acylcarnitines (tetradecadienylcarnitine, octadecenoylcarnitine, and octadecadienylcarnitine were found to be decreased upon IR exposure. Carnitine was present in the highest abundance followed by acetylcarnitine, propionylcarnitine, butyrylcarnitine, and valerylcarnitine respectively[65-67]. Conversely, increases of long-chain acylcarnitines at higher IR doses may be due to increased IR induced apoptosis of bone marrow during hematopoietic syndrome[68]. Carnitine, acetylcarnitine, and butyrylcarnitine were also found to increase in irradiated NHP urine with carnitine and acylcarnitine in urine which were much higher than were observed in serum[65]. Significant increase in lipid peroxidation biomarkers have been observed at post-irradiation[69]. Global profiling indicated an increase in polyunsaturated lipids attributed to enhance of arachidonic and docosahexaenoic acid[63].

5. CONCLUSIONS

Biomarkers are defined as the outcome of the repurcations associated with a particular disease and disorder. Specific biomarker is used to screen, diagnose, prognose, predict the disease initiation, progression and recurrence[70]. There are no set specific standards or criteria for development and validation of specific biomarker[71]. Exposure to ionising radiation (IR) can cause deleterious biological effects in humans (Fig. 4).

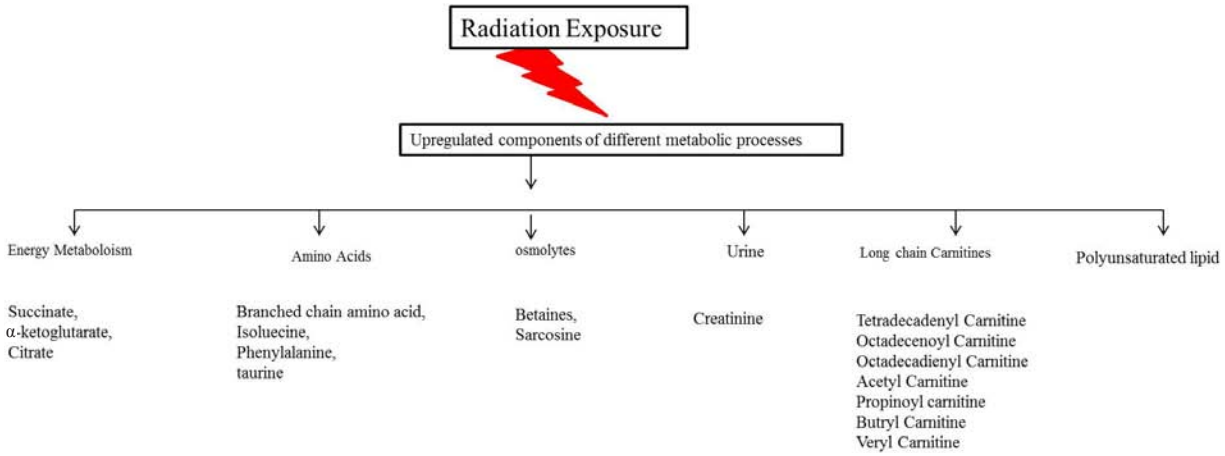

Figure 3. Radiation induced metabolic biomarkers. IR induced changes in components of urine, osmolytes, amino acids, components of energy metabolism, long chains carnitine and poly unsaturated lipids

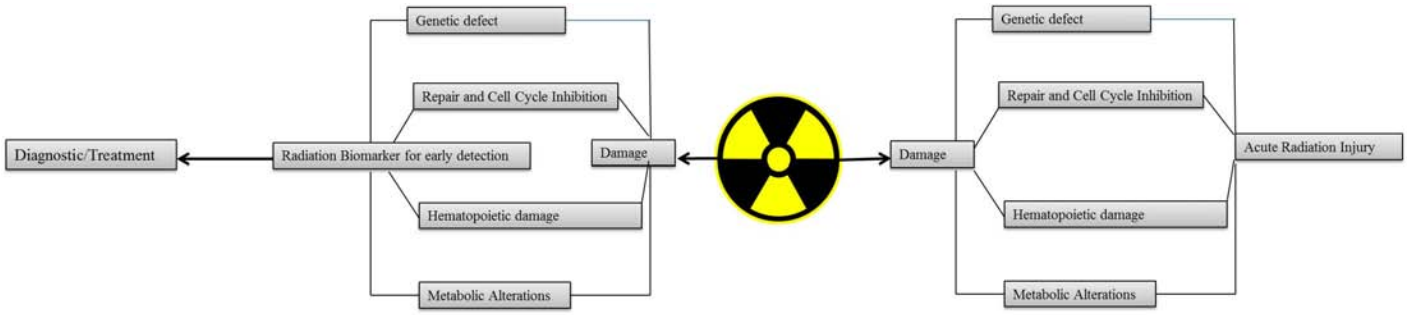

Figure 4. Concept image of radiation biomarker and its role in diagnostic and therapeutic. Graphical representation of radiation induced damage and changes in the system and role of biomarker in early detection and treatment.

Therefore, there is an urgent need to develop radiation biomarkers indicative of early and delayed whole body and organ-/tissue-specific injury that may facilitate the clinical management of afflicted populations.

6. SUMMARY

Radiation exposure has become an important concern for human beings in everyone's daily life, because a person may receive irradiation from many different sources. Hence a suitable biomarker to analyse and measure the pathological manifestations and response to therapeutic interventions are necessity. Biomarker could be of any type diagnostic or prognostic or predictive based upon our interest in assessing tissue injury. In the cellular milieu DNA is the critical target for radiation induced damage. The activation of the pathway related to DNA repair activates the pathway related to cell cycle check points and leading to apoptosis. Some of the cell cycle regulators like CDK, GADD45, Cyclin G1 and CHK-2 serve as the genetic biomarker. Amylase, Flt3 ligand, CRP and γ-H2AX are protein biomarker that provides the estimation of systemic inflammation. In the recent time several cytokine and chemokines have been identified as candidate biomarker for radiation injury such as IL-6, IL-18 and G-CSF. Chromosomal abbreviation induced by ionising radiation serves as biomarker for radiation exposure. Formation of Dicentrics, Premature chromosome condensation and formation of micronuclei are promising radiation biomarker for individual radiation sensitivity. In the current scenario metabolic biomarker have been identified by chemical profiling through NMR and Mass spectroscopy of biofluid that gives the dose dependent concentration profile of endogenous small molecules. Some of the know metabolites like lactate, alanine, glutamine, glutamate, alanine, α-ketoglutarate and 2-ketoisocarporate are validated metabolic biomarker.

7. FUTURE DIRECTION

Use of advanced mathematical, statistical, and computational modelling for evaluation of large datasets generated from multiple clinical and molecular parameters to understand radiation biology has already been established. Now technological advances in detection, acquisition, and processing have made the metabolomics platform a reliable source of data collection, there is still however, an urgent need for standardisation of protocols and analytical methods to validate these biomarkers and enable their use in the clinical or translational science settings. Careful and systematic collection, processing and storage of bio fluids are critical for downstream metabolomic studies for validation of research findings across institutions and for future systems biology analyses.

REFERENCES

1. Rana, S.; Kumar, R.; Sultana, S. & Sharma, R.K. Radiation-induced biomarkers for the detection and assessment of absorbed radiation doses. *J. Pharm. Bioallied. Sci.,* 2010, **2**, 189–196. doi: 10.4103/0975-7406.68500
2. Singh, V.K.; Newman, V.L.; Romaine, P.L.; Hauer-Jensen, M. & Pollard, H.B. Use of biomarkers for assessing radiation injury and efficacy of countermeasures. *Expert. Rev. Mol. Diagn.*, 2016, **16**(1), 65-81. doi: 10.1586/14737159.2016.1121102
3. Fenech, M. Current status, new frontiers and challenges in radiation biodosimetry using cytogenetic, transcriptomic and proteomic technologies. *Radiat. Measurements.*, 2011, **46**, 737–741. doi: 10.1016/j.radmeas.2011.01.016
4. Paul, S.; Barker, C.A.; Turner, H.C.; McLane, A.; Wolden, S.L. & Amundson, S.A. Prediction of in vivo radiation dose status in radiotherapy patients using ex vivo and in vivo gene expression signatures. *Radiat. Res.*, 2011, **175**, 257–265. doi: 10.1667/RR2420.1
5. Budworth, H.; Snijders, A.M.; Marchetti, F.; Mannion, B.; Bhatnagar, S.; Kwoh, E.; Tan, Y.; Wang, S.X.; Blakely, W.F.; Coleman, M.; Peterson, L. & Wyrobek, A.J. DNA repair and cell cycle biomarkers of radiation exposure and inflammation stress in human blood. *PLoS One.*, 2012, **7**(11), e48619. doi: 10.1371/journal.pone.0048619
6. De Zio, D.; Cianfanelli, V. & Cecconi, F. New insights into the link between DNA damage and apoptosis. *Antioxid. Redox Signal.*, 2013, **19**(6), 559-571. doi: 10.1089/ars.2012.4938
7. Melis, J.P.; van Steeg, H. & Luijten, M. Oxidative DNA damage and nucleotide excision repair. *Antioxid.Redox. Signal.* , 2013, **18**(18), 2409-2419. doi: 10.1089/ars.2012.5036
8. D'Errico, M.; Calcagnile, A.; Iavarone, I.; Sera, F.; Baliva, G.; Chinni, L.M.; Corona, R.; Pasquini, P. & Dogliotti, E. Factors that influence the dna repair capacity of normal and skin cancer affected individuals. *Canc. Epidemiol. Biomark. Preven.*, 1999, **8**, 553-559.
9. Amundson, S.A. & Fornace, A.J. Gene expression profiles for monitoring radiation exposure. *Radiat. Prot. Dosi.*, 2001, **97**, 11–16. doi: 10.1093/oxfordjournals.rpd.a006632
10. Zschenker, O.; Illies, T. & Ameis, D. Overexpression of lysosomal acid lipase and other proteins in atherosclerosis. *J. Biochem.*, 2006, **140**, 23–28. doi: 10.1093/jb/mvj137
11. Mayer, C.; Popanda, O.; Greve, B.; Fritz, E.; Illig, T.; Eckardt-Schupp, F.; Korn, R.; Gomolka, M.; Benner, A. & Schmezer, P. A radiation-induced gene expression signature as a tool to predict acute radiotherapy-induced adverse side effects. *Canc. Lett.*, 2011, **302**, 20–28. doi: 10.1016/j.canlet.2010.12.006
12. Brengues, M.; Paap, B.; Bittner, M.; Amundson, S.; Seligmann, B.; Korn, R.;Lenigk, R. & Zenhausern, F. Biodosimetry on small blood volume using gene expression assay. *Health Physics.*, 2010, **98**, 179–185. doi: 10.1097/01.HP.0000346706.44253.5c
13. Zhang, R.; Burns, F.J.; Chen, H.; Chen, S. & Wu, F. Alterations in gene expression in rat skin exposed to ^{56}Fe ions and dietary vitamin A acetate. *Radiat. Res.*, 2006,**165**, 570–581. doi: 10.1667/RR3556.1

14. Paul, S. & Amundson, S.A. Development of gene expression signatures for practical radiation biodosimetry. *Int. J.Radiat.Oncol. Biol. Phys.*, 2008, **71**, 1236–1244. doi: 10.1016/j.ijrobp.2008.03.043
15. Ding, L.H.; Park, S.; Peyton, M.; Girard, L.; Xie, Y.; Minna, J.D. & Story, M.D. Distinct transcriptome profiles identified in normal human bronchial epithelial cells after exposure to gamma-rays and different elemental particles of high Z and energy. *BMC Genomics*, 2013, **14**, 372. doi: 10.1186/1471-2164-14-372
16. Dressman, H.K.; ;Muramoto, G.G.; Chao, N.J.; Meadows, S.; Marshall, D.; Ginsburg, G.S.; Nevins, J.R. & Chute, J.P. Gene expression signatures that predict radiation exposure in mice and humans. *PLoS Med.*, 2007, **4**, e106. doi: 10.1371/journal.pmed.0040106
17. Gandhi, S.A.; Smilenov, L.B.; Elliston, C.D.; Chowdhury, M, & Amundson, S.A. Radiation dose-rate effects on gene expression for human biodosimetry. *BMC Med. Genomics.*, 2015, **8**, 22. doi: 10.1186/s12920-015-0097-x
18. Ruhm, W.; Woloschak, G.E.; Shore, R.E.; Azizova, T.V.; Grosche, B.; Niwa, O.; Akiba, S.; Ono, T.; Suzuki, K.; Iwasaki, T.; Ban, N.; Kai, M.; Clement, C.H.; Bouffler, S.; Toma, H.; Hamada, N. Dose and dose-rate effects of ionizing radiation: a discussion in the light of radiological protection. Radiat. *Environ.Biophys.*, 2015, **54**, 379-401. doi: 10.1007/s00411-015-0613-6
19. Rogakou, E.P.; Pilch, D.R.; Orr, A.H.; Ivanova, V.S. & Bonner, W.M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.*, 1998, **273**, 5858-5868. doi: 10.1074/jbc.273.10.5858
20. Rodrigue, A.; Lafrance, M.; Gauthier, M.C.; McDonald, D.; Hendzel, M. & West, S.C.; Jasin, M. & Masson, J.Y. Interplay between human DNA repair proteins at a unique double-strand break in vivo. *EMBO. J.*, 2006, **25**, 222-231. doi: 10.1038/sj.emboj.7600914
21. Wyman, C. & Kanaar, R. DNA double-strand break repair: all's well that ends well. *Annu. Rev. Genet.*, 2006, **40**, 363-383. doi: 10.1146/annurev.genet.40.110405.090451
22. Rothkamm, K. Different means to an end: DNA doublestrand break repair. *In* Life sciences and radiation, Edited by Kiefer, J. Berlin, Springer Verlag., 2004, 179-86. doi: 10.1007/978-3-642-18687-5_15
23. Schmid, T.E.; Zlobinskaya, O. & Multhoff, G. Differences in phosphorylated histone H2AX foci formation and removal of cells exposed to low and high linear energy transfer radiation. *Curr. Genomics.*, 2012, **13**, 418–425. doi: 10.2174/138920212802510501
24. Stiff, T.; O'Driscoll, M.; Rief, N.; Iwabuchi, K.; Löbrich, M. & Jeggo, P.A. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Canc. Res.*, 2004, **64**, 2390-2396. doi: 10.1158/0008-5472.CAN-03-3207
25. Furuta, T.; Takemura, H.; Liao, Z.Y.; Aune, G.J.; Redon, C.; Sedelnikova, O.A.; Pilch, D.R.; Rogakou, E.P.; Celeste, A.; Chen, H.T.; Nussenzweig, A.; Aladjem, M.I.; Bonner, W.M. & Pommier, Y. Phosphorylation of histone H2AX and activation of Mre11, Rad50, and Nbs1 in response to replication-dependent DNA double-strand breaks induced by mammalian DNA topoisomerase I cleavage complexes. *J. Biol. Chem.*, 2003, **278**, 20303-20312. doi: 10.1074/jbc.M300198200
26. Bertho, J.M.; Demarquay, C.; Frick, J.; Joubert, C.; Arenales, S.; Jacquet, N.; Sorokine-Durm, I.; Chau, Q.; Lopez, M.; Aigueperse, J.; Gorin, N.C.; Gourmelon, P. Level of Flt3-Ligand in Plasma: A possible new bio-indicator for radiation-induced aplasia. *Int. J. Radiat. Biol.*, 2001, **77**, 703-712. doi: 10.1080/09553000110043711
27. Ouellet-Morin, I.; Danese, A.; Williams, B. & Arseneault, L. Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain Behav. Immun.*, 2011, **25**, 640–646. doi: 10.1016/j.bbi.2010.12.020
28. Hayashi, N.; Nishimura, K. & Kumagai, S. New biomarkers for rheumatoid arthritis. *Rinsho Byori*., 2008, **56**, 297-308.
29. Singh, P.K.; Wise, S.Y.; Ducey, E.J.; Brown, D.S. & Singh, V.K. Radioprotective efficacy of tocopherol succinate is mediated through granulocyte-colony stimulating factor. *Cytokine.*, 2011, **56**, 411–421. doi: 10.1016/j.cyto.2011.08.016
30. Singh, V.K.; Brown, D.S. & Kao, T.C. Alpha tocopherol succinate protects mice from gamma-radiation by induction of granulocyte-colony stimulating factor. *Int. J. Radiat. Biol.*, 2010, **86**, 12–21. doi: 10.3109/09553000903264515
31. Singh, V.K.; Christensen, J.; Fatanmi, O.O.; Gille, D.; Ducey, E.J.;Wise, S.Y.; Karsunky, H. & Sedello, A.K. Myeloid progenitors: a radiation countermeasure that is effective when initiated days after irradiation. *Radiat. Res.*, 2012, **177**, 781–791. doi: 10.1667/RR2894.1
32. Krivokrysenko, V.I.; Shakhov, A.N. & Singh, V.K.; Bone, F.; Kononov, Y.; Shyshynova, I.; Cheney, A.; Maitra, R.K.; Purmal, A.; Whitnall, M.H.; Gudkov, A.V. & Feinstein, E. Identification of granulocyte colony-stimulating factor and interleukin-6 as candidate biomarkers of CBLB502 efficacy as a medical radiation countermeasure. *J. Pharmacol. Exp. Ther.*, 2012, **343**, 497–508. doi: 10.1124/jpet.112.196071
33. Ossetrova, N.I.; Condliffe, D.P.; Ney, P.H.; Krasnopolsky, K.; Hieber, K.P.; Rahman, A. & Sandgren, D.J. Early-response biomarkers for assessment of radiation exposure in a mouse total-body irradiation model. *Health Phys.*, 2014, **106**, 772–786. doi: 10.1097/HP.0000000000000094
34. Ossetrova, N.I.; Sandgren, D.J. & Blakely, W.F. b. Protein biomarkers for enhancement of radiation dose and injury assessment in nonhuman primate total-body irradiation model. *Radiat. Prot. Dosi.*, 2014, **159**, 61–76.

doi: 10.1093/rpd/ncu165
35. Redon, C.E.; Nakamura, A.J.; Martin, O.A. Nagy, V.; Parekh, P.R.; Weyemi, U.S. & Bonner, W.M. Recent developments in the use of gamma-H2AX as a quantitative DNA double-strand break biomarker. *Aging.*, 2011, **3**, 168–174.
doi: 10.18632/aging.100284
36. Blakely, W.F.; Sandgren, D.J.; Nagy, V.; Kim, S.Y.; Sigal, G.B. & Ossetrova, N.I. Further biodosimetry investigations using murine partial-body irradiation model. *Radiat. Prot. Dosi.*, 2014, **159**, 46–51.
doi: 10.1093/rpd/ncu127
37. Ha, C.T.; Li, X.H.; Fu, D.; Moroni, M.; Fisher, C.; Arnott, R.; Srinivasan, V. & Xiao, M. Circulating interleukin-18 as a biomarker of total body radiation exposure in mice, minipigs, and nonhuman primates (NHP). *PLoS One.*, 2014, **9**, e109249.
doi: 10.1371/journal.pone.0109249
38. Hu, S.; Blakely, W.F. & Cucinotta, F.A. HEMODOSE: a biodosimetry tool based on multi-type blood cell counts. *Health Phys.*, 2015, **109**, 54–68.
doi: 10.1097/HP.0000000000000295
39. Singh, V.K.; Romaine, P.L. & Seed, T.M. Medical countermeasures for radiation exposure and related injuries: characterization of medicines, FDA-approval status and inclusion into the strategic national stockpile. *Health. Phys.*, 2015, **108**, 607– 630.
doi: 10.1097/HP.0000000000000279
40. IAEA, Cytogenetic Dosimetry: Applications in preparedness for and response to radiation emergencies, 2011.
41. Cardis, E.; Kesminiene, A.; Ivanov, V.; Malakhova, I.; Shibata, Y.; Khrouch, V.; Drozdovitch, V.;, Maceika, E.; Zvonova, I.; Vlassov, O.; Bouville, A.; Goulko, G.; Hoshi, M.; Abrosimov, A.; Anoshko, J.; Astakhova, L.; Chekin, S.; Demidchik, E.; Galanti, R.; Ito, M.; Korobova, E.; Lushnikov, E.; Maksioutov, M.; Masyakin, V.; Nerovnia, A.; Parshin, V.; Parshkov, E.; Piliptsevich., N.; Pinchera, A.; Polyakov, S.; Shabeka, N.; Suonio, E.; Tenet, V.; Tsyb, A.; Yamashita, S. & Williams, D. Risk of thyroid cancer after exposure to 131I in childhood. *J. Natl. Cancer Inst.*, 2005, **97**, 724–732.
doi: 10.1093/jnci/dji129
42. Kodama, Y.; Pawel, D.; Nakamura, N.; Preston, D.; Honda, T.; Itoh, M.; Nakano, M.; Ohtaki, K.; Funamoto, S. & Awa, A.A. Stable chromosome aberrations in atomic bomb survivors: results from 25 years of investigation. *Radiat. Res.*, 2001, **156**, 337–346.
doi: 10.1667/0033-7587(2001)156[0337:SCAIAB]2.0.CO;2
43. Darroudi, F. & Natarajan, A.T. Cytogenetical characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. VII. Complementation analysis of X-irradiated wild-type CHO-K1 and xrs mutant cells using the premature chromosome condensation technique. *Mutat. Res*., 1989, **213**, 249–255.
doi: 10.1016/0027-5107(89)90157-7
44. Prasanna, P.G.S. & Blakely, W.F. Premature chromosome condensation in human resting peripheral blood lymphocytes for chromosome aberration analysis using specific whole-chromosome DNA hybridization probes. *Methods Mol. Biol.*, 2005, **291**, 49–57.
doi: 10.1385/1-59259-840-4:049
45. Lamadrid, A.I.; Garcı´a, O.; Delbos, M.; Voisin, P. & Roy, L. PCC-ring induction in human lymphocytes exposed to gamma and neutron irradiation. *J. Radiat. Res.*, 2007, **48**,1–6.
doi: 10.1269/jrr.0625
46. Lindholm, C.; Stricklin, D.; Jaworska, A.; Koivistoinen, A.; Paile, W.; Arvidsson, E. Deperas-Standylo, J.; Wojcik, A. Premature chromosome condensation (PCC) assay for dose assessment in mass casualty accidents. *Radiat. Res.*, 2010, **173**, 71–78.
doi: 10.1667/RR1843.1
47. Ainsbury, E.A.; Bakhanova, E.; Barquinero, J.F.; Brai, M.; Chumak, V.; Correcher, V.; Darroudi, F.; Fattibene, P.; Gruel, G.; Guclu, I.; Horn, S.; Jaworska, A.; Kulka, U.; Lindholm, C.; Lloyd, D.; Longo,.; Marrale, M.; Monteiro, Gil, O.; Oestreicher, U.; Pajic, J.; Rakic, B.; Romm, H.; Trompier, F.; Veronese, I.; Voisin, P.; Vral, A.; Whitehouse, C.A.; Wieser, A.; Woda, C.; Wojcik, A. & Rothkamm, K. Review of retrospective dosimetry techniques for external ionising radiation exposures. *Radiat. Prot. Dosi.*, 2011, **147**, 573–592.
doi: 10.1093/rpd/ncq499
48. Blasco, M.A. Telomeres and human disease: ageing, cancer and beyond. *Nat. Rev. Genet.*, 2005, **6**, 611–622.
doi: 10.1038/nrg1656
49. Williams, E.S.; Klingler, R.; Ponnaiya, B.; Hardt, T.; Schrock, E.; Lees-Miller, S.P.; Meek, K.; Ullrich, R.L. & Bailey, S.M. Telomere dysfunction and DNA-PKcs deficiency: characterization and consequence. *Canc. Res.*, 2009, **69**, 2100–2107.
doi: 10.1158/0008-5472.CAN-08-2854
50. Ayouaz, A.; Raynaud, C.; Heride, C.; Revaud, D.& Sabatier, L. Telomeres: Hallmarks of radiosensitivity. *Biochimie.*, 2008, **90**, 60–72.
doi: 10.1016/j.biochi.2007.09.011
51. Castella, M.; Puerto, S.; Creus, A.; Marcos, R. & Surralles, J. Telomere length modulates human radiation sensitivity *in vitro*. *Toxicol. Lett.*, 2007, **172**, 29–36.
doi: 10.1016/j.toxlet.2007.05.012
52. M'kacher, R.; Bennaceur-Griscelli, A.; Girinsky, T.; Koscielny, S.; Delhommeau, F.; Dossou, J.; Violot, D.; Leclercq, E.; Courtier, M.H.; Beron-Gaillard, N.; Assaf, E.; Ribrag, V.; Bourhis, J.; Feneux, D.; Bernheim, A.; Parmentier, C. & Carde, P. Telomere shortening and associated chromosomal instability in peripheral blood lymphocytes of patients with Hodgkin's lymphoma prior to any treatment are predictive of second cancers. *Int. J. Radiat. Oncol. Biol. Phys.*, 2007, **68**, 465–471.
doi: 10.1016/j.ijrobp.2007.01.050
53. Williams, J.P. & McBride, W.H. After the bomb drops: a new look at radiation-induced multiple organ dysfunction syndrome (MODS). *Int. J. Radiat. Biol.*, 2011, **87**, 851-868.

doi: 10.3109/09553002.2011.560996
54. Rossnerova, A.; Spatova, M.; Schunck, C. & Sram, R.J. Automated scoring of lymphocyte micronuclei by the MetaSystemsMetafer image cytometry system and its application in studies of human mutagen sensitivity and biodosimetry of genotoxin exposure. *Mutagenesis*, 2011, **26**, 169–175. doi: 10.1093/mutage/geq057
55. Fenech, M. Cytokinesis-block micronucleus cytome assay. *Nat. Protoc.*, 2007, **2**, 1084–1104. doi: 10.1038/nprot.2007.77
56. Surowy, H.; Rinckleb, A.; Luedeke, M.; Stuber, M.; Wecker, A.; Varga, D.; Maier, C.; Hoegel, J. & Vogel, W. Heritability of baseline and induced micronucleus frequencies. *Mutagenesis.*, 2011, **26**, 111–117. doi: 10.1093/mutage/geq059
57. Tyburski, J.B.; Patterson, A.D.; Krausz, K.D.; Slavı´k, J.; Fornace, A.J. Jr; Gonzalez, F.J. & Idle, J.R. Radiation metabolomics. Identification of minimally invasive urine biomarkers for gamma-radiation exposure in mice. *Radiat. Res.*, 2008, **170**(1), 1–14. doi: 10.1667/RR1265.1
58. Khan, A.R.; Rana, P.; Tyagi, R.; Kumar, I.P.; Devi, M.M.; Javed, S. & Khushu, S. NMR spectroscopy based metabolic profiling of urine and serum for investigation of physiological perturbations during radiation sickness. *Metabolomics.*, 2011, **7**, 583–592. doi: 10.1007/s11306-011-0277-4
59. Sundgren, P.C.; Nagesh, V.; Elias, A.; Tsien, C.; Junck, L.; Hassan, G.D.M.; Lawrence, T.S.; Chenevert, T.L.; Rogers, L.; McKeever, P. & Cao, Y. Metabolic alterations: a biomarker for radiation-induced injury of normal brain. An MR Spectroscopy study. *J. Magn. Reso. Imaging*, 2009, **29**, 291–297. doi: 10.1002/jmri.21657
60. Martin, F.P.; Wang, Y.; Sprenger, N.; Yap, I.K.; Lundstedt, T.; Lek, P.; Rezzi, S.; Ramadan, Z.; van Bladeren, P.; Fay, L.B.; Kochhar, S.; Lindon, J.C.; Holmes, E. & Nicholson, J.K. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol. Syst. Biol.*, 2008, **4**, 157. doi: 10.1038/msb4100190
61. Zhang, Y.; Zhou, X.; Li, C.; Wu, J.; Kuo, J.E. & Wang, C. Assessment of early triage for acute radiation injury in rat model based on urinary amino acid target analysis. *Mol. Biosyst.*, 2014, **10**, 1441-1449. doi: 10.1039/C3MB70526A
62. Menon, S.S.; Uppal, M.; Randhawa, S.; Cheema, M.S.; Aghdam, N.; Usala, R.L.; Ghosh, S.P.; Cheema, A.K. & Dritschilo, A. Radiation metabolomics: current status and future directions. *Front Oncol.*, 2016, **6**, 20. doi: 10.3389/fonc.2016.00020
63. Broin, P.O.; Vaitheesvaran, P.; Saha, S.; Hartil, C.; Chen, E.I.; Goldman, D.; Fleming,W.H.; Kurland, I.J.; Guha, C. & Golden, A. Intestinal microbiota derived metabolomic blood plasma markers for prior radiation injury. *Int. J. Radiat. Oncol. Biol. Phys.*, 2016, **91**, 360–367. doi: 10.1016/j.ijrobp.2014.10.023
64. Waters, N.J.; Waterfield, C.J.; Farrant, R.D.; Holmes, E. & Nicholson, J.K. Integrated metabonomic analysis of bromobenzene-induced hepatotoxicity: novel induction of 5-oxoprolinosis. *J. Proteome. Res.* , 2006, **5**, 1448-1459. doi: 10.1021/pr060024q
65. Pannkuk, E.L.; Laiakis, E.C.; Authier, S.; Wong, K. & Fornace, A.J. Global Metabolomic Identification of Long-Term Dose-Dependent Urinary Biomarkers in Nonhuman Primates Exposed to Ionizing Radiation. *Radiat Res.*, 2015, **184**, 121–133. doi: 10.1667/RR14091.1
66. Houten, S.M. & Wanders, R.J. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. *J. Inherit. Metab. Dis.*, 2010, **33**, 469–477. doi: 10.1007/s10545-010-9061-2
67. Jones, C.M.; Goh, V.; Sebag-Montefiore, D. & Gilbert, D.C. 5Biomarkers in anal cancer: from biological understanding to stratified treatment. *Br. J. Canc.*, 2017, **116**, 156–162. doi: 10.1038/bjc.2016.398
68. Macia, J.; Regot, S.; Peeters, T.; Conde, N.; Sole, R. & Posas, F. Dynamic signaling in the Hog1 MAPK pathway relies on high basal signal transduction. *Sci. Signal.*, 2009, **24**, 63. doi: 10.1126/scisignal.2000056
69. Yoshida, Y.; Umeno, A. & Shichiri, M. Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity in vivo. *J. Clin. Biochem. Nutr.*, 2013, **52**(1), 9–16. doi: 10.3164/jcbn.12-112
70. Nalejska & Ewelina. Prognostic and predictive biomarkers. *Mol. Oncol. Genetic.*, 2014, **18**, 273–284. doi: 10.1007/s40291-013-0077-9
71. Craig-Schapiro, R.; Fagan, A.M. & Holtzman D.M. Biomarkers of Alzheimer's disease. *Neurobiol Dis.*, 2009, **35**(2), 128-140. doi: 10.1016/j.nbd.2008.10.003

CONTRIBUTORS

Dr Saurabh Mishra is a Research Fellow at Radiation Biotechnology Group at Institute of Nuclear Medicine and Allied Sciences. He received his PhD in Biochemistry from Jamia Hamdard and has published several articles on radiation induced oxidative stress.

Dr Raj Kumar has received his PhD Biotechnology from IIT, Roorkee. Currently he is working as Scientist 'E' and heading the Radiation Biotechnology Group at Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi. He is involved in development of novel drug candidates from secondary metabolites isolated from radioresistant bacteria and has extensive knowledge in the field of radiation biology, oxidative stress, nutraceuticals and radiation countermeasure agents.

Contribution in current study, guidance, planning the experiments, analysing results and critical reviewing of the manuscript.