Radiation Biomarkers: Applications in Triage Management of Radiation Victims

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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.

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.

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 cyogenetic damage in blood lymphocytes. Limitations of these methods are they are time-consuming and thus not suitable for triage exposed persons during radiation eventuality.

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). 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. Another major pathway to repair bulky DNA damages is Nucleotide excision repair (NER) that repairs helical distortion. The proteins of NER are involved in repairing oxidative damage through activation of BER that includes
protein 2 (DDB2) radiation, including XPC and Damage Specific DNA binding protein 2 (DDB2)\textsuperscript{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)\textsuperscript{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\textsuperscript{14}. The ex vivo model of human blood irradiation has been used to investigate the early radiation induced biological responses for potential biodosimetry applications\textsuperscript{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\textsuperscript{14-16}. However, there is very less information regarding co-exposure of confounding factors that can affect the utility of individual biomarker of radiation biodosimetry\textsuperscript{14}. The ex vivo model of human blood irradiation has been used to investigate the early radiation induced biological responses for potential biodosimetry applications\textsuperscript{4}. There is a study that reports about the transcriptional response of well-known DNA repair, cell cycle and apoptosis regulating genes after irradiation.

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 (RB3 and GRM2) and in another case, where genes were down regulated by LDR only but not with acute doses (DUSP3 and ID2, Fig. 3)\textsuperscript{9}. 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\textsuperscript{26}. 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\textsuperscript{27}. However, the change in gene expression beyond 4.45 Gy was higher in the acute exposure group as compared to LDR exposed groups of cells.

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\textsuperscript{21,22}. A linear correlation was established in double strand breaks (DSBs) and radiation dose. It has been reported that 1.0 Gy of radiation induces approximately 20-40 DSBs per cell nucleus\textsuperscript{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 radiation\textsuperscript{24}. Whereas, ATR and DNA-PK appear to be involved in γ-H2AX formation at the sites of replication-associated breaks\textsuperscript{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\textsuperscript{26}. 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. Both of these parameters are relatively easy to analyse quantitatively in serum/plasma using a clinical blood chemistry analyser or using ELISA. 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.

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. 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. 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 lyz-amylose are some prominent members of radiation sensitive proteins biomarkers. Previously, interleukin-18 (IL-18) has been reported up-regulated at 5–10 Gy and is considered as bio-indicator of bone marrow damage. IL-18 serum concentrations are also correlated with radiation dose in mini-pigs, NHPs, and mice.

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. 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.

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, 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 be 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. 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 proliferating cells. 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. PCC technique has been considered as the most useful technique for assessing high dose acute exposures of low LET radiation. 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).

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. 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. It is has been reported earlier that short telomeres participates in inducing genomic instability in the aged progeny of irradiated cells. The telomere length modulates chromosome radiosensitivity in healthy individuals. The analysis of the telomere lengths in the study of persons exposed to radiation shows potential to exist as a radiation biomarker.

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. Such analysis could be made on fresh blood or frozen peripheral blood lymphocytes. Micronuclei analysis by cytogenesis-blocked micronucleus (CBMN) assay exhibits a great potential as a biomarker for individual’s radiosensitivity.

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. 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 $^1\text{H}$ NMR (proton NMR). Profile of urine samples revealed the presence of lactate, alanine, glutamine and alanine some other metabolites like hippurate, taurine, trimethyl amine (TMA), $\alpha$-ketoglutarate ($\alpha$-KG) and 2-ketoisocaproate were also present (Fig. 3). NMR profiling of serum of irradiated mice was characterized by the predominance of lipids along with creatine, amino acids and organic acids. Comparative analysis revealed about the significant changes were observed mainly in metabolites associated with energy metabolism (succinate, $\alpha$-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. 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. On the other hand, metabolic changes observed in the serum were branched chain amino acid, lipids and lactate. Some of the membrane metabolites viz., choline and phosphoethanolamine were increased only during post irradiation. 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. 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. Interestingly, longer chain acylcarnitines (tetradecadecylcarnitine, octadecenoylcarnitine, and octadecadecylcarnitine were found to be decreased upon IR exposure. Carnitine was present in the highest abundance followed by acetylcarnitine, propionylcarnitine, butyrylcarnitine, and valerylcarnitine respectively. Conversely, increases of long-chain acylcarnitines at higher IR doses may be due to increased IR induced apoptosis of bone marrow during hematopoietic syndrome. 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. Significant increase in lipid peroxidation biomarkers have been observed at post-irradiation. Global profiling indicated an increase in polyunsaturated lipids attributed to enhance of arachidonic and docosahexaenoic acid.

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. There are no set specific standards or criteria for development and validation of specific biomarker. Exposure to ionising radiation (IR) can cause deleterious biological effects in humans (Fig. 4).
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, Fli3 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.

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