Defence Science Journal, Vol. 61, No. 2, March 2011, pp. 133-137, DOI: 10.14429/dsj.61.833

Predictive Genomics: A Post-genomic Integrated Approach to Analyse Biological Signatures of Radiation Exposure

Manikandan Jayapal[*], Swaminathan Sethu[*], Dimphy Zeegers[*], Birendranath Banerjee[*#], and M. Prakash Hande[*†]

[*]*Yong Loo Lin School of Medicine, National University of Singapore, Singapore-117 597*
[#]*KIIT School of Biotechnology, KIIT University, Bhubaneswar-751 024*
[†]*E-mail: phsmph@nus.edu.sg*

ABSTRACT

The ultimate objective of radiation research is to link human diseases with the altered gene expression that underlie them and the exposure type and level that caused them. However, this has remained a daunting task for radiation biologists to indent genomic signatures of radiation exposures. Transcriptomic analysis of the cells can reveal the biochemical or biological mechanisms affected by radiation exposures. Predictive genomics has revolutionised how researchers can study the molecular basis of adverse effects of exposure to ionising radiation. It is expected that the new field will find efficient and high-throughput means to delineate mechanisms of action, risk assessment, identify and understand basic mechanisms that are critical to disease progression, and predict dose levels of radiation exposure. Previously, we have shown that cells responding to environmental toxicants through biological networks that are engaged in the regulation of molecular functions such as DNA repair and oxidative stress. To illustrate radiation genomics as an effective tool in biological dosimetry, an overview has been provided of some of the current radiation genomics landscapes as well as potential future systems to integrate the results of radiation response profiling across multiple biological levels in to a broad consensus picture. Predictive genomics represents a promising approach to high-throughput radiation biodosimetry.

Keywords: Biomarkers, ionising radiation, genomics, microarray, genomic integration, biodosimetry, predictive genomics, radiation research, genomic signatures, transcriptomic analysis

1. INTRODUCTION

In an escalating alarm about the possible accident or an attack using radiologic or nuclear devices, several countries have established an emergency support group to assess and prioritise needs for a response to such an event. One such high-priority establishment in the United States is of new biomarker approach to biodosimetry[1]. This approach offers the possibility to measure interactions in a miniaturised, economic, automated, and qualitative or quantitative way providing insights into the cellular machinery of diverse organisms. This application is not only restricted to the security and military sector but it can also be used in the fields of medical diagnostics or public health. In a major radiologic emergency, estimating exposure of doses and adverse effects would be a daunting task, especially with the current methods using dosimeters. In recent years, functional genomics approaches, such as global transcriptomic profiling, have been developed to simultaneously monitor changes in gene expression across the whole genome[2]. As genome-wide expression signatures have become increasingly accessible, the quantity of information on transcriptomic responses to ionising radiation has increased considerably. While several individual studies have provided insight into many aspects of radiation response, the variety of experimental models and dose/time-exposure experiments, sophisticated data analysis methods, and genomic integration, are still being developed. Regulating the gene expression upon ionising radiation is a fundamental mechanism by which cells employ the information in their DNA. Prior to high throughput genomics, early DNA damage responsive genes identified from studies focusing on individual genes, pathways, or biological processes[3-4]. Such methods were continued to be adopted as technical advancements has allowed us to use global genomic expression profiling in a multi-parametric analysis and statistical and data-mining approaches have become more advanced[5].

2. CURRENT RADIATION GENOMICS LANDSCAPE

With the complete sequencing of the genome and the availability of commercial platforms, global transcriptomic analyses have become routine tools. One of the first microarray experiments, investigating response to ionising radiation exposure for 4 h after 2 Gy gamma-rays in human cells, found 1,344 genes in a myeloid cancer cell line[6]. This includes approximately 30 novel radiation-sensitive genes that were never been reported previously. A similar transcriptomic profiling was employed to detect the potential biomarkers in peripheral blood exposed to doses between

Received 21 October 2010, Revised 21 November 2010, Published Online 13 February 2011

0.2 Gy and 2 Gy of gamma radiation[7]. One of the significant studies that attempted to find relationship between high and low doses was carried out by Yin[8], *et al.* The authors have profiled gene expression signatures of mouse brain exposed to 0.1 Gy and 2 Gy at 0.5 and 4 h post-exposure time points. This study has revealed unique genes that are differentially regulated at low dose but not at higher dose**.** An interesting functional study by Wang[9], *et al.* identified 75 genes as possible contributors to low dose hypersensitivity in a human lymphoblast cell line after 0.5 Gy irradiation. Gene expression changes were also validated by RNAi approach by specifically knocking down a gene called CDHC to investigate its role in cell death at low doses. Another study by Svensson[10], *et al.* showed transcriptomic profiling for defining gene sets of late toxicity in radiotherapy patients.

Transcriptomic profiling was performed on peripheral blood lymphocytes of patients with or without late radiation toxicity. The blood lymphocytes were stimulated to divide in culture and exposed to 2 Gy x-rays and microarray analysis were performed 24 h later. Instead of single genes, set of genes were used to classify the expression profiles. This result was consistent with those from previous *in vivo* studies. Amundson[11], *et al.* carried out the first transcriptomic profiling in the NCI-60 cells and they have identified 22 genes associated with low survival after 2 Gy gamma rays, 14 genes associated with low survival after 8 Gy, and 25 genes with radiation responses dependent on wild-type p53. E2F4 and RBL2 are the only 2 genes that were commonly down-regulated in all the 63 of the cell lines studied. It has been proposed that these two genes could be a potential target for radiotherapy. However, the authors also suggest that the basal gene expression pattern before irradiation may be a better predictor of radiation sensitivity. A recent study by Paul and Amundson[12] showed genome-wide transcriptomic signatures for radiation biodosimetry. This approach could provide dose estimation as well as adverse risk assessment. Human peripheral blood from 10 healthy donors was irradiated *ex vivo* and genome-wide expression analysis approach was applied on both 6 h and 24 h after exposure. Microarray analysis revealed 74-gene signature that distinguishes between four radiation doses (0.5 Gy, 2 Gy, 5 Gy, and 8 Gy) and controls. While genome-wide transcriptomic profiling has proven to be valuable in the prediction of biomarkers of radiation exposure, one must recognise that transcriptional changes do not necessarily correlate with protein expression. To elucidate pathogenesis of disease, it is important to know the genes and the protein involved. The application of biological network analysis has allowed to integrate literature-based analysis for the generation of experimentally feasible hypotheses in radiation biology[13-14].

3. PROPOSED MODEL

While several studies revealed the fine details of both high-dose and low-dose specific transcriptional responses to ionising radiation, a consistent broad consensus picture remains indefinable. There is huge amount of data generated by the radiation genomics studies and it is important to incorporate such information into concise and meaningful models of radiation response profiling across multiple biological levels. The purpose of the proposed model would be to confirm and assess sudden/accidental radiation exposure in the shortest possible time frame. This can be achieved by the following:

(i) The management of a large microarray data set in a coordinated and integrated manner.
(ii) Performing feature extraction and dimensionality reduction to find specific genes and/or combinations of genes indicative of exposure to a specific radiologic or nuclear agent.
(iii) Designing a classifier that can determine if a cell has been exposed to a known agent and dose.

The condensed platform should cover information on a particular agent (for example, radiation) and its dose, mechanism of action, gene networks, pathways, and any kind of radiation genomics data that is available. It is also important to make possible correlation of existing results obtained from transcriptomic, proteomic, and metabolomic profiles. Data generated from omics technologies in the context of dose, time, target tissue, and phenotypic severity across a range of species, from yeast, to nematode, to human, will provide the comparative information needed to assess the severity of exposure. As human risk estimation is important, the *in vitro* assays corresponding to the key processes being monitored *in vivo* will enable the validation and quantitative relationship between assay results and any particular *in vivo* system[15].

Similar integrated approach has been used by pharmaceutical companies, for instance, Iconix's DrugMatrix. The DrugMatrix application possess data sets for the systemic effects of hundreds of drugs on different rat tissues and it has been claimed by them to have potential for the identification of toxicities of new chemical entities[16-17]. With the Iconix's ToxFX application, one can submit gene expression data and predict toxicological assessment of the test compound. However, there are several other possible genomic signatures that might be used including epigenetic patterns, microRNA profiles, disease associations, protein expression and metabolite profiles. Such signatures should be generated from high throughput screening and these will provide strong explanation for observed findings**.** Unifying knowledge from public data repositories such as ArrayExpress, Gene Expression Omnibus (GEO), Gene Expression Atlas, Oncomine and other existing literatures will provide a comprehensive compendium. These mentioned areas should be explored to advance the efficacy and accuracy of integrated system to facilitate advancements:

The proposed model (Fig. 1) will have the following salient features:

- Comprehensive modules like epigenetic and miRNA profiles along with transcriptomic profiles, since epigenetic factors and miRNA are increasingly associated with a variety of disease pathogenesis.

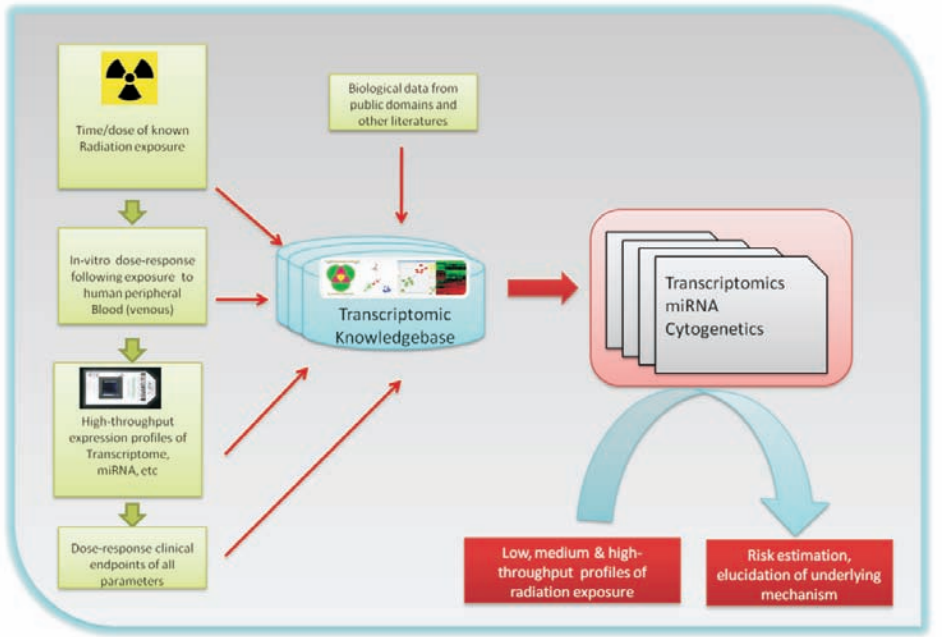


Figure 1. Schematic representation of integrated ionising radiation knowledge base.

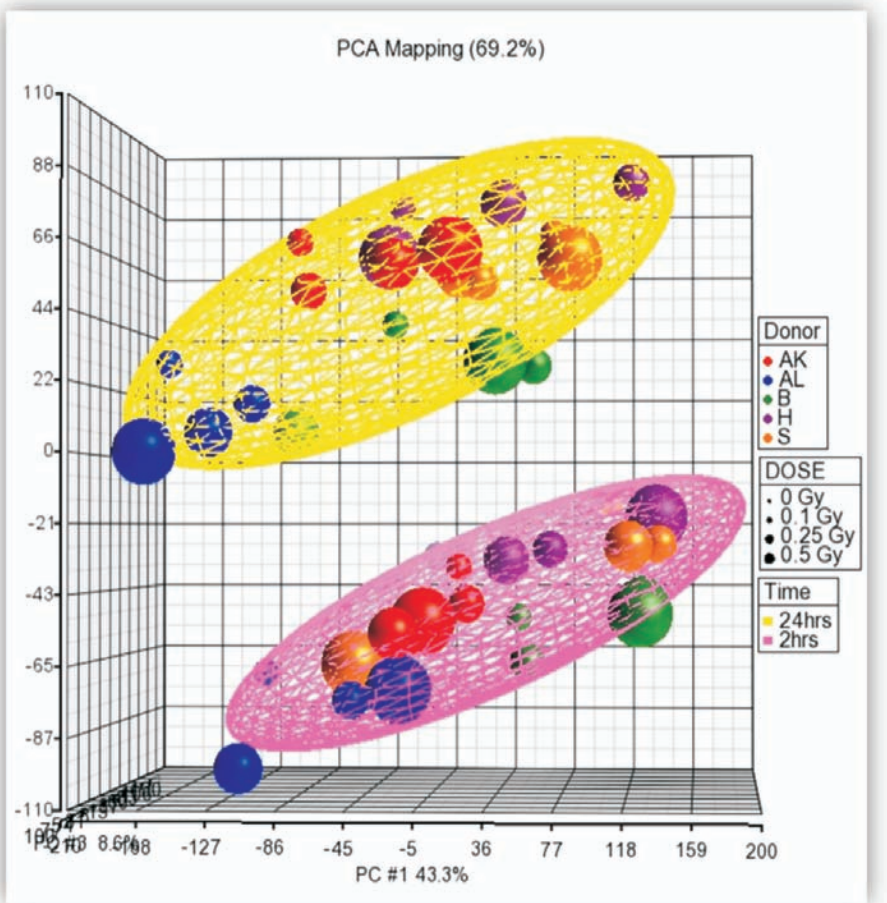


Figure 2. Principle component analysis of transcriptomic profile of human peripheral blood lymphocytes following exposure to ionising radiation.

- A wide spectrum platform to assess adverse effects in major organs and species using different mammalian cell culture sources.
- Cross-module integration to assess severity of damage and understand the underlying mechanism of clinical end points.

However, the proposed model does have its own limitations. Conceivably, a large number of parameters would need to be optimised for each perturbation, including cell type, agent, dose, and time.

Genomic analysis in radiation biology provides an opportunity to change and improve the way it is currently investigated. In an ongoing study, the authors have employed an integrated approach as mentioned in the model (Fig. 1). Along with the conventional end points such as cytogenetic markers, gene expression profiling of irradiated blood samples was also done for biomarker identification. An example of such data is shown in Fig. 2, where differential gene expression profiling is shown for human blood lymphocytes irradiated ex vivo to different doses of gamma radiation at 2 h and 24 h post-exposure. Currently, the authors are performing thorough analysis of this data to identify potential gene signatures following irradiation. The identification of ionising radiation response markers through the sensible use of genomics not only helps in predicting exposure dose and time , but also promises more accurate diagnosis and risk assessment, leading to more precise prognosis and new therapeutic interventions[18]. As the accessibility of methods for obtaining complete high-throughput measurements of RNA continues to increase, more information on genome-wide responses to ionising radiation will become available. It is important to integrate the results across biological levels to build models that can describe the underlying mechanism for radiation responses (Fig. 1). Such a model would allow prediction of clinical end points to a radiation exposure challenge, thus enabling personalisation of treatment regimens or radiation risk estimation.

The schematic representation (Fig. 1) depicts various sources of transcriptomic knowledge base. The source includes known ionising radiation exposure dose/time frames, transcriptomic and microRNA profiling signatures, clinical end points as well as biological data from literature. Any ionising radiation exposure profiles can be queried against the knowledge base for identifying time/dose frames, type of agent and risk estimation.

The representation (Fig.2) indicates the principle component analysis based clustering and sub-clustering of the various data points including number of donors (AK, AL, B, H & S), doses (0, 0.1, 0.25 and 0.5) and post-irradiation time points (2 h and 24 h).

ACKNOWLEDGEMENTS

This work was supported by the grants from Defence Innovative Research Programme, Defence Science and Technology Agency, Singapore (POD: 0613592, WBS: 185-000-150-422). The authors thank Ms Prarthana Srikanth, Ms Swapna Nandakumar, and Ms Pallavi Srivastava for their help during the study. Dr Veena Hande is thanked for critically reading the manuscript.

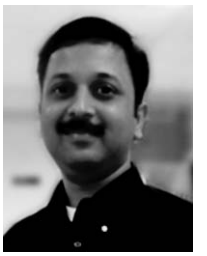
REFERENCES

1. Pellmar, T.C. & Rockwell, S. Priority list of research areas for radiological nuclear threat countermeasures. *Radiation Research,* 2005, **163**, 115-23.
2. Jayapal, M. & Melendez, A.J. DNA microarray technology for target identification and validation. *Clin. Exp. Pharmacol. Physiol.,* 2006, **33**, 496-503.
3. Sheikh, M.S.; Carrier, F.; Papathanasiou, M.; Hollander, M.; Zhan, Q.; Yu, K. & Fornace, A.J. Identification of several human homologs of hamster DNA damage-inducible transcripts. Cloning and characterization of a novel UV-inducible cDNA that codes for a putative RNA-binding protein. *J. Biol. Chem.*, 1997, **272**, 26720–726.
4. Bae, I.; Smith, M.; Sheikh, M.S.; Zhan, Q.; Scudiero, D.; Friend, S.H.; O'connor, P. & Fornace, A.J.Jr. An abnormality in the p53 pathway following g-irradiation in many wild-type p53 human melanoma lines. *Cancer Research*, 1996, **56,** 840-47.
5. Weinstein, J. Spotlight on molecular profiling: Integromic analysis of the NCI-60 cancer cell lines. *Mol. Cancer. Ther.,* 2006, **5**, 2601-605.
6. Amundson, S.; Bittner, M.; Chen, Y.; Trent, J.; Meltzer, P. & Fornace, A.J.Jr. cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress responses. *Oncogene*, 1999,**18**, 3666-672.
7. Amundson, S.; Shahab, S.; Bittner, M.; Meltzer, P.; Trent, J. & Fornace, A.J.Jr. Identification of potential mRNA markers in peripheral blood lymphocytes for human exposure to ionizing radiation. *Radiation Research*, 2000, **154**, 342-46
8. Yin, E.; Nelson, D.; Coleman, M.; Peterson, L. & Wyrobek, A. Gene expression changes in mouse brain after exposure to low dose ionizing radiation. *Int. J. Radiat. Biol.,* 2003, **79**, 759-75.
9. Wang, H.; Long, X.; Sun, Z.; Rigaud, O.; Xu, Q.; Huang, Y.; Sui, J.; Bai, B. & Zhou, P. Identification of differentially transcribed genes in human lymphoblastoid cells irradiated with 0.5 Gy of gamma-ray and the involvement of low dose radiation inducible CHD6 gene in cell proliferation and radiosensitivity. *Int. J. Radiat. Biol.,* 2006, **82**, 181-90.
10. Svensson, J.; Stalpers, L.; Esveldt-Van, L.R.E.; Franken, N.; Haveman, J.; Klein, B.; Turesson, I.; Vrieling, H. & Giphart-Gassler, M. Analysis of gene expression using gene sets discriminates cancer patients with and without late radiation toxicity. *PLoS Med.*, 2006, **3**, 1904-914.
11. Amundson, S.A.; Do, K.T.; Vinikoor, L.C.; Lee, R.A.; Koch-Paiz, C.A.; Ahn, J.; Reimers, M.; Chen, Y.; Scudiero, D.A.; Weinstein, J.N.; Trent, J.M.; Bittner, M.L.; Meltzer, P.S. & Fornace, A.J.Jr. Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institute Anticancer Drug

Screen. *Cancer Research*, 2006, 68, 415-24.
12. Paul, S. & Amundson, S.A. Development of gene expression signatures for practical radiation biodosimetry. *Int. J. Radiat. Oncol. Biol. Phys.*, 2008, **15**(71), 1236-244.
13. Jayapal M, Bhattacharjee RN, Melendez AJ, Hande MP. Environmental toxicogenomics: a post-genomic approach to analysing biological responses to environmental toxins. *Int. J. Biochem. Cell Biol.*, 2010, **42**, 230-40.
14. Nikitin, A.; Egorov, S.; Daraselia, N. & Mazo, I. Pathway studio—The analysis and navigation of molecular networks. *Bioinformatics*, 2003; 19:2155–157
15. Edwards, S.W. & Preston, R.J. Systems biology and mode of action based risk assessment. *Toxicology Science*, 2008, **106**, 312-8.
16. Ganter, B.; Tugendreich, S.; Pearson, C.I.; Ayanoglu, E.; Baumhueter, S.; Bostian, K.A.; Brady, L.; Browne, L.J.; Calvin, J.T.; Day, G.J.; Breckenridge, N.; Dunlea, S.; Eynon, B.P.; Furness, L.M.; Ferng, J.; Fielden, M.R.; Fujimoto, S.Y.; Gong, L.; Hu, C.; Idury, R.; Judo, M.S.; Kolaja, K.L.; Lee, M.D.; McSorley, C.; Minor, J.M.; Nair, R.V.; Natsoulis, G.; Nguyen, P.; Nicholson SM.; Pham H.; Roter AH.; Sun D.; Tan S.; Thode S.; Tolley AM.; Vladimirova A.; Yang, J.; Zhou, Z. & Jarnagin, K. Development of a large-scale chemogenomics database to improve drug candidate selection and to understand mechanisms of chemical toxicity and action. *Journal of Biotechnology*, 2005, **119**, 219-44.
17. Fielden, M.R.; Eynon, B.P.; Natsoulis, G.; Jarnagin, K.; Banas, D. & Kolaja, K.L. A gene expression signature that predicts the future onset of drug-induced renal tubular toxicity. *Toxicol Pathol.*, 2005, **33**, 675-83.
18. Barcellos-Hoff, M.H. & Costes, S.V. A systems biology approach to multicellular and multi-generational radiation responses. *Mutation Research*, 2006, **597**, 32-38.

Contributors

Mr Manikandan Jayapal obtained his PhD on Mast Cell Genomics from the School of Medicine, National University of Singapore, Singapore. Previously, he was a Research Fellow in the Department of Physiology, School of Medicine, NUS from 2002-2009. His current research is focused on the biomarker analysis using Next Generation Sequencing analysis, RNA interference, micro RNAs and other cutting-edge molecular biotechniques in the fields of cancer, immunology and neurobiology.



Dr Swaminathan Sethu received his PhD from National University of Singapore, Singapore, for his work on inflammatory roles of Sphingosine kinase and Phospholipase D, and carried out postdoctoral studies on the telomerase inhibition in the management of cancer. Currently, he is Research Fellow at Genome Stability Laboratory, Department of Physiology, School of Medicine, NUS, Singapore. His current research is to study the effects of low doses of different types of radiations on normal human cells to assess and predict the potential long and short term health risks in humans.

Ms Dimphy Zeegers received her Master's degree from the Utrecht University in the Netherlands with specialisation in cancer genomics. She is currently working as a Research Assistant at the Genome Stability Laboratory, Department of Physiology, NUS, Singapore.

Dr Birendranath Banerjee received his PhD from SVYASA University and Manipal Hospital, Bengaluru in the field of Radiation Biology and cancer. He did part of his Doctoral work and Post-doctoral work at National University of Singapore (NUS). He is currently an Assistant Professor at the School of Biotechnology, KIIT University, Bhubaneswar, India. His research interest includes Stress-induced DNA damage response and molecular cytogenetics.

Dr M. Prakash Hande obtained his PhD in Radiobiology in 1991 from the Kasturba Medical College, Manipal. He is currently an Associate Professor in the Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore. He was a Post-doctoral Fellow in the Department of Radiation Genetics and Chemical Mutagenesis, at the then Sylvius Laboratories, Leiden University, The Netherlands, during 1994-1997. He then took up the post-doctoral scientist position at the Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, Canada, to work in the field of Telomere Biology. He has established the fact that DNA damage response and repair proteins are involved in telomere maintenance mechanisms in mammalian cells. Currently, his research is focused on the following: DNA repair factors and telomeres, oxidative damage and telomeres, experimental therapeutics–with special emphasis on telomerase inhibition in cancer cells, toxicogenomics–biological response markers of exposure to environmental pollutants including radiation.