Letter to the Editor

Identification and Validation of Candidate Radiation-responsive Genes for Human Biodosimetry*

LI Shuang, LU Xue, FENG Jiang Bin, TIAN Mei, and LIU Qing Jie#

The aim of the present study is to analyze the global research trend of radiation-responsive genes and identify the highly reproducible radiation-responsive genes. Bibliometric methods were applied to analyze the global research trend of radiation-responsive genes. We found 79 publications on radiation-responsive genes from 2000 to 2017. A total of 35 highly reproducible radiation-responsive genes were identified. Most genes are involved in response to DNA damage, cell proliferation, cell cycle regulation, and DNA repair. The p53 signal pathway was the top enriched pathway. The expression levels of 18 genes in human B lymphoblastoid cell line (AHH-1) cells were significantly up-regulated in a dose-dependent manner at 24 h after exposure to 0-5 Gy ^{60}Co γ-ray irradiation. Our results indicate that developing a gene expression panel with the 35 high reproducibility radiation-responsive genes may be necessary for qualitative and quantitative assessment after exposure.

Key words: Gene expression; Biodosimetry; Bibliometric; Ionizing radiation; Dose estimation

Ionizing radiation is an extremely ubiquitous and significant environmental hazard that has been identified as both public health and national security risks. Exposure to ionizing radiation in daily life is usually low, but radiation accidents and incidents can cause significant exposure[1]. In a large-scale radiological event or nuclear exposure incident, thousands of individuals may be exposed. Rapidly distinguishing exposed from non-exposed individuals and estimating of the doses received by individuals are crucial to guide the appropriate trial and clinical medical treatment[2]. In the absence of physical dosimeter, biological dosimetry can be used to estimate the dose after radiation exposure[3].

In the past few years, gene expression alteration following ionizing radiation exposures has been shown to potentially function as a convenient, rapid and high-throughput radiation biodosimetry in a large-scale radiological emergency[4-6]. The expression levels of different candidate genes were regulated in a dose- and time-dependent manner after exposure to ionizing radiation, which have been identified in whole blood[7], isolated lymphocytes[8], and T cells[9] using microarrays or quantitative real-time polymerase chain reaction (qRT-PCR). Most studies have suggested that gene expression changes in human peripheral blood cells, particularly lymphocytes, can be used as a sensitive tool to assess radiation exposure. Furthermore, high-throughput platforms for peripheral blood RNA isolation combined with 384-well low-density arrays qRT-PCR or DNA microarrays techniques represent a new dimension in high sampling capacity. However, monitoring specific single gene expression to assess the absorbed dose in response to radiation exposure is limited. Therefore, developing a radiation-specific gene expression panel with high reproducibility may be necessary for covering a range of doses, dose-rates, and time-points to improve accuracy and robustness of dose estimation.

In the present study, bibliometric analysis was employed to qualitatively and quantitatively assess global research from 2000 to 2017. The radiation-responsive genes with high reproducibility studied in the inclusive literature were found to possess consistent dose-effect relationships in more than three independent studies. To determine the significant dose-effect relationship of these genes after radiation exposure, we validated the expression of radiation-responsive genes expression in human B lymphoblastoid cell line (AHH-1) at certain time points following exposure to ^{60}Co γ-rays using qRT-PCR.

Data Retrieval Data were obtained from Pubmed/Medline, Embase, and the Web of Science

doi: 10.3967/bes2017.112

*This work was financially supported by the National Natural Science Foundation of China [Nos. 81172593, 81573081].

China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing 100088, China

databases. To improve retrieval accuracy, Medical Subject Headings (MeSH) terms were used to search both on the Pubmed/Medline and Embase database. Pubmed/Medline was searched as follows: 'gene expression' [MeSH Terms] or ('gene' [All Fields] and 'expression' [All Fields]) or 'gene expression' [All Fields], and biodosimetry [All Fields]; Embase was searched as follows: 'gene'/exp or gene and ('expression'/exp or expression) and ('biodosimetry'/exp or 'biodosimetry'). For bibliometric analysis, the Web of Science Core Collection is the most widely used source of scientific information. In this study, the terms 'gene expression' and 'biodosimetry' were used to retrieve titles, key words, author information, abstracts, and reference from the Web of Science. The period of publication was limited from 2000 to 2017.

Inclusive and Exclusive Criteria Inclusive and exclusive criteria were as follows: (1) peer-reviewed articles were published and indexed in three databases, including original research articles, meeting abstracts, and proceedings, excluding reviews, editorial material, and books. Citation databases were as follows: Science Citation Index Expanded (1900-present); Social Sciences Citation Index (1990-present); Arts and Humanities Citation Index (1990-present); Conference Proceedings Citation Index-Science (1990-present); Conference Proceedings Citation Index-Social Science and Humanities (1990-present); Emerging Sources Citation Index (2015-present). (2) Inclusion of the publications should include a summary of the information that can provide sufficient information, or wherein full-text information can be obtained. (3) Species were not limited. (4) Repeated publications from different database sources were excluded. (5) Articles that were inconsistent with the purpose of the study were also excluded.

Bibliometric Analysis Results from the Web of Science were exported and analyzed with Microsoft Excel 2010, including the type of literature, annual publication output, journal publication, distribution of publication by subject area, countries and institutions, and top published authors of studies on ionizing radiation-induced gene expression alteration. To further explore the most-cited articles, CiteSpace VI (64 bits) was used to construct co-citation references network. CiteSpace VI (64 bits) is a visualization tool that can analyze trends and patterns in scientific literature and detect critical points in the development of a field[10].

Screening the Radiation-responsive Genes All the ionizing radiation-induced differential expression genes from inclusive literature were listed and analyzed with Microsoft Excel 2010. The inclusive genes possessed an obvious dose-effect relationship after exposure to radiation in at least one independent study. The highly reproducible radiation-responsive genes studied in the inclusive literature were found to possess consistent dose-effect relationships in more than three independent studies.

Bioinformatics Analysis Gene Ontology (GO) enrichment (http://gather.genome.duke.edu/) was applied to biologically classify the genes. Then, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.kegg.jp/) was used to map the differential genes to the possible molecular pathways. STRING (http://string.embl.de/) and Cytoscape 3.4.0 (http://www.cytoscape.org/) were employed to build protein interaction networks onto which radiation-responsive genes expression can be mapped.

Cell Culture Previous studies have proved that lymphocytes are both sensitive to early radiation injury and highly responsive in terms of induced gene expression changes. In this study, AHH-1 cells were obtained from American Type Culture Collection (Manassas, VA, USA). AHH-1 cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% heat-inactivated fetal bovine serum (HyClone; GE Healthcare Life Sciences, Logan, UT, USA), 2 mmol/L L-glutamine (Thermo Fisher Scientific, Inc.), 100 U/mL penicillin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 100 μg/mL streptomycin (Sigma-Aldrich; Merck KGaA). The AHH-1 cell line is diploid and its population-doubling time ranges between 16 and 19 h. AHH-1 cells were incubated at 37 ˚C in a humidified 5% CO_2 atmosphere.

Sample Irradiation Irridiation with ^{60}Co γ-rays was performed in the Beijing Radiation Center (Beijing, China). The source radioactivity was 130 TBq. The exposure setup was calibrated by physical measurement using a tissue-equivalent ionizing chamber. The radiation dose rate was calculated using the source radioactivity and the distance between the source and sample: a dose-rate of 1 Gy/min corresponded to a source-sample distance of 73.5 cm. The homogeneous irradiation field was 30 cm × 30 cm; the samples were placed within a 5 cm radius circle and the uncertainty of the calibration was 1%. AHH-1 cells were seeded in flasks (1×10^7 cells/mL) and irradiated. Radiation doses 0, 1, 3, and