Absence of mutations in the human interferon alpha-2b gene in workers chronically exposed to ionising radiation

Dauren Botbayev1,2,3, Gloria Ravegnini2, Giulia Sammarini2, Polat Kazymbet4, Elisabetta Cilli5, Patrizia Serventi5, Alexandra Khanseitova1, Bakhytzhan Alzhanuly4, Ayaz Belkozhaev1, Nagima Aitkhozhina1, Meirat Bakhtin4, Vittorio Lodi6, Patrizia Hrelia3, and Sabrina Angelini3

1 Aitkhozinh Institute of Molecular Biology and Biochemistry KS MES, Almaty, Kazakhstan
2 Al-Farabi Kazakh National University, Almaty, Kazakhstan
3 Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy
4 Institute of Radiobiology and Radiation Protection, Medical University, Astana, Kazakhstan
5 Laboratories of Physical Anthropology and Ancient DNA, Department of Cultural Heritage, University of Bologna, Ravenna Campus, Italy
6 Occupational Health Unit, Sant’Orsola-Malpighi Hospital, Bologna, Italy

[Received in September 2018; Similarity Check in September 2018; Accepted in March 2019]

Individuals chronically exposed to low-level ionising radiation (IR) run the risk of harmful and long-term adverse health effects, including gene mutations and cancer development. The search for reliable biomarkers of IR exposure in human population is still of great interest, as they may have a great implementation potential for the surveillance of occupationally exposed individuals. In this context, and considering previous literature, this study aimed to identify mutations in the human interferon alpha-2b (hIFNα-2b) as a potential biomarker of occupational chronic low-dose IR exposure linking low-IR exposure to the effects on haematopoiesis and reduced immunity. The analysis was performed in the genomic DNA of 51 uranium miners and 38 controls from Kazakhstan, and in 21 medical radiology workers and 21 controls from Italy. hIFNα-2b gene mutations were analysed with the real-time polymerase chain reaction (PCR) or Sanger sequencing. However, none of the investigated workers had the hIFNα-2b mutation. This finding highlights the need for further research to identify biomarkers for early detection of health effects associated with chronic low-dose IR exposure.

KEY WORDS: hIFNα-2b mutations; genotoxicity; radiology workers; uranium miners

In addition to cellular DNA damage and damage response, exposure to ionising radiation (IR) triggers non-targeted effects mainly related to the immune system (1–4). Two recent studies reported an association between mutations in the human interferon alpha-2b (hIFNα-2b) gene and chronic exposure to low-level of IR in medical personnel (5) and residents from Pakistan (6). The IFNα-2b gene encodes a protein belonging to the class of cytokines with immunomodulatory, antiviral, anti-proliferative, and anti-tumour activities (7–10). Mutations, typically the frameshift ones or single nucleotide changes in the human IFNα-2b gene could therefore compromise the functioning of the immune system. In addition, these mutations have been detected in brain tumour patients exposed to different environmental stressors, including high levels of naturally occurring IR (10), and other cancer patients (11, 12). We considered these findings interesting because of the health consequences associated with chronic exposure to low doses of IR. As mutations in the human IFNα-2b gene might compromise immunity, we wondered if they could also serve as biomarkers for health monitoring of occupational and environmental chronic exposure to low IR doses. The aim of this study was therefore to verify the hypothesis in a group of uranium miners from Kazakhstan and in a group of Italian radiology workers.

MATERIALS AND METHODS

Study population

Volunteer coal miners, all men (n=51) were recruited from two Kazakh regions, Aksu and Zavodskoy (Northern Kazakhstan). In Aksu the mean effective dose is 4 mSv/year, while in Zavodskoy it is 4.95 mSv/year (13). Figure 1 shows a map of the area with the sources of IR exposure. Matched controls (n=38) were anonymous blood donors recruited at the Almaty Blood Donation Centre. Formal written consent was signed by all participants before inclusion in the study.
The Italian study arm consisted of 42 hospital workers, 21 of whom were occupationally exposed to IR and 21 were healthy, unexposed controls. These participants represent a subset of our previous study in radiological workers (14), whose DNA samples had been stored for future sub-studies. They had signed informed consent for the use of their samples in sub-studies such as this, according to Helsinki Declaration and its later amendments.

Both the Kazakh and Italian participants were taken 10 mL of blood in heparinised tubes during regular medical examination. Blood samples were stored at -80 °C until DNA isolation and subsequent IFNα-2b mutation analysis.

Genomic DNA was isolated from frozen peripheral blood lymphocytes using a standardised phenol-chloroform extraction method. The DNA was purified with 70 % ethanol, air dried overnight at room temperature, and re-suspended in approximately 100 µL of deionised nuclease-free water. Before we analysed the samples for mutations, we selected ten IFNα-2b mutations appearing at the frequency higher than 5 %, as reported by Shahid et al. (5, 6), which, according to these authors, were associated with chronic low-dose IR exposure and its potential to induce point mutations in single genes and modulate the expression of a variety of genes (15–17).

We then analysed our samples for these ten mutations using real-time polymerase chain reaction (PCR) with a customised Applied Biosystems™ Taqman Assay® (Thermo Fisher Scientific, Waltham, MA, USA) as recommended by manufacturer or using Sanger sequencing with an ABI Prism™ 310 Genetic Analyzer (Thermo Fisher Scientific) as described by Shashid et al. (5) (Table 1). Blanks were included in each reaction for quality control.

RESULTS AND DISCUSSION

Surprisingly, none of the analysed samples had any of the ten mutations of this gene. Reasons for our negative findings may be several. One of them is a possible bias in the selection of mutations. Instead of sequencing the entire coding region of the hIFNα-2b gene, we selected ten most frequent mutations identified by Shahid et al. (5, 6). Another

Table 1 Characteristics of the IFNα-2b gene mutation

| Mutation (base position)| Frequency* | Methods       |
|-------------------------|------------|---------------|
| C insertion (8–9)       | 5.4 %      | Real-time PCR |
| A to T (187)            | 8 %        | Real-time PCR |
| T to A (219)            | 8 %        | Real-time PCR |
| A to G (256)            | 8 %        | Real-time PCR |
| C insertion (330–331)   | 8 %        | Real-time PCR |
| A deletion (435)        | 3.3 %      | Sanger sequencing |
| G to A (436)            | 4.3 %      | Sanger sequencing |
| A to G (437)            | 3.3 %      | Sanger sequencing |
| A deletion (439)        | 4.3 %      | Sanger sequencing |
| A deletion (477)        | 3.3 %      | Real-time PCR |

* Frequency reported by Shahid et al (5, 6); † gene accession number NM_006055

Figure 1 IR sources of exposure at locations of the Kazakh study arm
CONCLUSIONS

Despite their promising potential, none of the available cytogenetic tests has become part of routine biodosimetry surveillance of occupationally and/or environmentally exposed individuals. These tests are traditionally manual and labour-intensive, even with the recently proposed automation protocol for MN and chromosomal aberration analysis (37, 38). Despite our enthusiasm about the promising potential of the IFNα-2b gene as a reliable biomarker of IR-associated immunological risk (5, 6), we did not observe any IFNα-2b gene mutation or changes in blood cell counts.

Unfortunately, we do not have any cytogenetic data (i.e. chromosome aberrations, MN, or the comet assay findings) or data about health consequences associated with low-dose radiation for the Kazakh study arm. In conclusion, our failure to find mutation in the IFNα-2b gene in either arm calls for further research that would identify reliable biomarkers for early detection of health effects associated with low-dose IR.

REFERENCES

1. Godekmerdan A, Ozden M, Ayar A, Gursu FM, Ozan AT, Serhatlioglu S. Diminished cellular and humoral immunity in workers occupationally exposed to low levels of ionizing radiation. Arch Med Res 2004;35:324–8. doi: 10.1016/j.arcmed.2004.04.005
2. Oskouii MR, Refahi S, Pourissa M, Tabarzadeh AS. Assessment of humoral immunity in workers occupationally exposed to low levels of ionizing radiation. Life Sci J 2013;10:58–62.
3. Rödel F, Frey B, Multhoff G, Gaipl U. Contribution of the immune system to bystander and non-targeted effects of ionizing radiation. Cancer Lett 2015;356:105–13. doi: 10.1016/j.canlet.2013.09.015
4. Voos P, Fuch S, Weipert F, Babel L, Tandl D, Meckel T, Hehlgans S, Fournier C, Moroni A, Rödel F, Thiel G. Ionizing radiation induces morphological changes and immunological modulation of Jurkat cells. Front Immunol 2018;9:922. doi: 10.3389/fimmu.2018.00922
5. Shahid S, Mahmood N, Nawaz Chaudhry M, Sheikh S, Ahmad N. Mutations of the human interferon alpha-2b (hIFNα2b) gene in occupationally protracted low dose radiation exposed personnel. Cytokine 2015;73:181–9. doi: 10.1016/j.cyto.2015.02.008
6. Shahid S, Mahmood N, Nawaz Chaudhry M, Ahmad N. Mutations of the human interferon alpha-2b (hIFNα2b) gene in low-dose natural terrestrial ionizing radiation exposed dwellers. Cytokine 2015;76:294–302. doi: 10.1016/j.cyto.2015.05.011
7. Parmar S, Platanis LC. Interferons: mechanisms of action and clinical implications. Curr Opin Oncol 2003;15:431–9. PMID: 14624225
8. Brandacher G, Winkler C, Schroeksnadel K, Margreiter R, Fuchs D. Antitumoral activity of interferon-gamma involved in impaired function in cancer patients. Curr Drug Metab 2006;7:599–612. doi: 10.2174/138920006778017768
9. Bose A, Baral R. IFNα2b stimulated release of IFNγamma differentially regulates T cell and NK cell mediated tumor cell cytotoxicity. ImmunoLett 2007;108:68–77. doi: 10.1016/j.imlet.2006.10.002

10. Levin D, Schneider WM, Hoffmann HH, Yarden M, Busetto AG, Manor O, Sharma N, Rice CM, Schreiber G. Multifaceted activities of type I interferon are revealed by a receptor antagonist. Sci Signal 2014;7:ra50. doi: 10.1126/scisignal.2004998

11. Shahid S, Nawaz Chaudry M, Mahmood N, Sheikh S. Mutation of the human interferon alpha-2b gene in brain tumor patients exposed to different environmental conditions. Cancer Gene Ther 2015;22:246–61. doi: 10.1038/cgt.2015.12

12. Shahid S, Nawaz Chaudhry M, Mahmood N. Mutations of the human interferon alpha-2b (hfIFNo-2b) gene in cancer patients receiving radiotherapy. Am J Cancer Res 2015;5:2455–66. PMCID: PMC4567871

13. Kazymbet PK, Bahkhit MM, Imasheva BS. Population radiation level of the north Kazakhstan by natural sources of ionized radiation. Astana Med J 2006;1:26-8.

14. Maffei F, Angelini S, Forti GC, Lodi V, Mattioli S, Hrelia P. Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors. Mutagensis 2002;17:405–9. doi: 10.1039/mu17.5.405

15. Trott K, Rosemann M. Molecular mechanisms of radiation carcinogenesis and the linear, non-threshold dose response model of radiation risk estimation. Radiat Environ Biophys 2000;39:79–87. PMID: 1029376

16. Gadhia P, Shah N, Nabata S, Patel S, Patel K, Pitathwala M, Tamakwula D. Cytogenetic analysis of radiotherapeutic and diagnostic workers occupationally exposed to radiations. Int J Human genet 2004;4:65. doi. 10.1080/09723757.2004.11885872

17. Jin YW, Na YJ, Lee YJ, An S, Lee JE, Jung M, Kim H, Nam SY, Kim CS, Yang KH, Kim SU, Kim WK, Park WY, Yoo KY, Kim CS, Kim JH. Comprehensive analysis of time-and dose-dependent patterns of gene expression in a human mesenchymal stem cell line exposed to low-doses ionizing radiation. Oncol Rep 2008;19:135–44. doi: 10.3892/or.19.1.135

18. Kazakh Ministry of Justice, Centre of Legal Information. О Стратегическом плане Агентства Республики Казахстан по атомной энергии на 2012 – 2016 годы [Strategic Plan of the Atomic Energy Agency of the Republic of Kazakhstan for 2012–2016, in Russian]. [displayed 27 March 2019]. Available at http://www.adilet.zan.kz/rus/docs/P1200001806/links

19. Angelini S, Kumar R, Carbone F, Maffei F, Cantelli-Forti G, Violante FS, Lodi V, Curti S, Hemminki K, Hrelia P. Micronuclei in humans induced by exposure to low level of ionizing radiation: influence of polymorphisms in DNA repair genes. Mutat Res 2005;570:105-17. doi: 10.1016/j.mrfmm.2004.10.007

20. Milić M, Rozgaj R, Kašuba V, Jazbec AM, Hrelia P, Angelini S. The influence of individual genome sensitivity in DNA damage repair assessment in chronic professional exposure to low doses of ionizing radiation. In: Chen CC, editor. Selected topics in DNA repair. London: IntechOpen; 2011 [displayed 27 March 2019]. Available at https://www.intechopen.com/books/selected-topics-in-dna-repair/the-influence-of-individual-genome-sensitivity-in-dna-damage-repair-assessment-in-chronic-profession

21. Fenech M, Knaasmulier S, Bolognesi C, Bonassi S, Holland N, Migliore L, Palitti F, Natarajan AT, Kirsch-Volders M. Molecular mechanisms by which in vivo exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes in vivo and ex vivo in humans. Mutat Res 2016;770:12–25. doi: 10.1016/j.mrrev.2016.04.008

22. Angelini S, Bermejo JL, Ravegnini G, Sammarini G, Hrelia P. Application of the lymphocyte Cytosine-Block Micronucleus Assay to population exposed to petroleum and its derivatives: results from a systematic review and meta-analysis. Mutat Res 2016;770:58–72. doi: 10.1016/j.mrrev.2016.03.001

23. Siama Z, Zosang-Zaali M, Vanlalruati A, Jagetia GC, Pau KS, Kumar NS. Chronic low dose exposure of hospital workers to ionizing radiation leads to increased micronuclei frequency and reduced antioxidants in their peripheral blood lymphocytes. Int J Radiat Biol 2019. doi: 10.1080/09553002.2019.1571255. [Epub ahead of print]

24. Khisroon M, Khan A, Naseem M, Ali N, Khan S, Rasheed SB. Evaluation of DNA damage in lymphocytes of radiology personnel by comet assay. J Occup Health 2015;57:268–74. doi. 10.1539/joh.14-0154-0A

25. Botbayev D, et al. Absence of mutations in the human interferon alpha-2b gene in workers chronically exposed to ionising radiation. Arh Hig Rada Toksikol 2019;70:104-108
Izostanak mutacija humanoga interferon alfa-2b gena u radnika kronično izloženih ionizirajućem zračenju

Kronična izloženost niskim razinama ionizirajućeg zračenja povezana je s rizikom od dugoročnih štetnih posljedica za zdravlje, što obuhvaća i mutacije gena te nastanak raka. U tijeku je potraga za pouzdanim biopokazateljima izloženosti ionizirajućem zračenju u ljudi, budući da njihova primjena može značajno unaprijediti praćenje profesionalno izloženih osoba. U tom smislu, a s obzirom na ranija saznanja, cilj je ovoga istraživanja bio utvrditi mutacije gena za proizvodnju humanoga interferona alfa-2b (hIFNα-2b gena) kao mogućega biopokazatelja profesionalne kronične izloženosti niskim dozama ionizirajućeg zračenja, koje je isto povezano s djelovanjem na hematopoezu i pad imuniteta. Analiziran je genomski DNA 51 rudara u rudnicima uranija te 38 kontrolnih ispitanika iz Kazahstana, odnosno genomski DNA 21 zdravstvenoga radnika na radiologiji i 21 kontrolnoga ispitanika iz Italije. Mutacije hIFNα-2b gena utvrđivane su metodom lančane reakcije polimerazom u stvarnom vremenu (engl. real-time PCR) odnosno sekvenciranjem prema Sangeru, ali se pokazalo da niti jedan radnik nije imao niti jednu od deset traženih mutacija toga gena. Stoga ne preostaje drugo nego i dalje tražiti pouzdane biopokazatelje za rano otkrivanje štetnih zdravstvenih učinaka povezanih s kroničnom izloženosti niskim dozama ionizirajućeg zračenja.

KLJUČNE RIJEČI: hIFNα-2b; genotoksičnost; radiologija; rudari, uranij; zdravstveni radnici

Botbayev D, et al. Absence of mutations in the human interferon alpha-2b gene in workers chronically exposed to ionising radiation Arh Hig Rada Toksikol 2019;70:104-108

32. Gaetani S, Monaco F, Bracci M, Ciaramica V, Impollonia G, Valentino M, Tomasetti M, Santarelli L, Amati M. DNA damage response in workers exposed to low-dose ionizing radiation. Occup Environ Med 2018;75:724–9. doi: 10.1136/ oemed-2018-105094
33. Maffei F, Angelini S, Forti GC, Violante FS, Lodi V, Mattioli S, Hrelia P. Spectrum of chromosomal aberrations in peripheral lymphocyte of hospital workers occupationally exposed to low doses of ionizing radiation. Mutat Res 2004;547:91–9. doi: 10.1016/j.mrfmm.2003.12.003
34. Tawn EJ, Curwen GB, Jonas P, Gillies M, Hodgson L, Cadwell KK. Chromosome aberrations determined by FISH in radiation workers from the Sellafield nuclear facility. Radiat Res 2015;184:296–303. doi: 10.1667/RR14125.1
35. Djokovic-Davidovic J, Milovanovic A, Milovanovic J, Antic V, Gajic M. Analysis of chromosomal aberrations frequency, haematological parameters and received doses by nuclear medicine professionals. J BUON 2016;21:1307–15. PMID: 27837637
36. Tawn EJ, Curwen GB, Riddle AE. Chromosome aberrations in workers occupationally exposed to tritium. J Radiol Prot 2018;38:N9–16. doi: 10.1088/1361-6498/aab0d0
37. Shirley B, Li Y, Knoll JHM, Rogan PK. Expedite radiation biodosimetry by automated dicentric chromosome identification (ADCI) and dose estimation. J Vis Exp 2017;127:56245. doi: 10.3791/56245
38. Lenzi M, Cocchi V, Hrelia P. Flow cytometry vs optical microscopy in the evaluation of the genotoxic potential of xenobiotic compounds. Cytometry B Clin Cytom 2018;94:696–706. doi: 10.1002/cyto.b.21546