Mutation Research 784-785 (2016) 1–7

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Lymphocytes with multiple chromosomal damages in a large cohort of West Siberia residents: Results of long-term monitoring

Vladimir Druzhinin[a,b], Maria Bakanova[b], Aleksandra Fucic[c,*], Tatiana Golovina[a], Yana Savchenko[b], Maxim Sinitsky[a], Valentin Volobaev[a]

[a] Kemerovo State University, Kemerovo, Russia
[b] Federal State Budget Scientific Institution «The Federal Research Center of Coal and Coal Chemistry of Siberian Branch of the Russian Academy of Sciences», Kemerovo, Russian Federation
[c] Institute for Medical Research and Occupational Health, Zagreb, Croatia

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 26 October 2015
Accepted 14 December 2015
Available online 18 December 2015

Keywords:
Rogue cell
Radon
In vivo irradiation
High LET radiation
Lymphocyte
Chromosome aberration
Double minute

ABSTRACT

Cells with specific multiple chromosome aberrations, defined as rogue cells (RC) have been described in different populations, predominantly those exposed to radiation. The frequency, etiology and related health risks have still not been elucidated due to their low frequency of occurrences and rarely performed studies. This study reports RC frequency using chromosome aberration (CA) assay in peripheral lymphocytes in the group of 3242 subjects, during a 30-year long follow-up study in a general rural and urban population, children environmentally exposed to radon, occupationally exposed population and lung cancer patients from the Kemerovo region (Siberia, Russian Federation). Results show that the highest RC frequency was present in children environmentally exposed to radon and the lowest in the general urban population. Total frequency of CA did not correlate with frequency of RC. Genotoxic analysis of air and water samples excluded anthropogenic pollution as a possible cause of genome damage and RC frequency. In 85% of RCs, double minutes, observed in a large number of human tumors, were present. Results of CA analysis suggested that radon and its decay products (alpha-emitters) were the leading factors causing RC in subjects exposed to high LET radiation. Thus, RC may be a candidate biomarker for exposure to this type of radiation.

1. Introduction

Among the large number of variouscytogenetic abnormalities observed in short-term cultures of lymphocytes from subjects exposed to ionizing radiation or in *in vitro* experiments, metaphases having numerous structural chromosome damages are of particular interest. Such metaphases, termed by A. Awa and J. Neel as "rogue cells" (RCs), have a very specific spectrum of abnormalities: multiple dicentric and even tricentric chromosomes, as well as numerous chromosome fragments, many with the appearance of "double minutes" [1]. RCs were first described nearly half a century ago [2,3] in peripheral blood lymphocytes extracted from South American Indians; researchers explained the occurrence of such cells as a result of an unknown tropical infection. Contagious diseases as a cause of RCs formation were discussed in connection with the detection of an increased concentration of antibodies to polyomavirus (JCV, BKV) [4–7]. Another origin of RC induction, associated with contagious diseases, was the possibility that certain enzymes are extracted from destroyed bacteria, which are not subject to proteolysis in a cytoplasm and are able to induce multiple various types of chromosome damage [8]. The hypothesis about RCs as a cytological effect of a failure in the cell cycle control process and abnormal apoptosis was also suggested [4]. However, many investigations performed in human populations from different countries have not shown a significant relationship between the phenomenon of RCs and contagious diseases [1–10]. Reports on RC frequency in control groups in comparison with groups exposed by well-known clastogens are contradictory [11–14]. Based on RC detection in groups of survivors of the atomic bombing of Hiroshima, liquidators of the Chernobyl atomic power plant, as well as residents of areas contaminated by radionuclides, it was suggested that RC induction is connected with ionizing radiation [15–20], in particular high LET radiation from internal contamination by α-particles [21–23].

* Corresponding author at. Institute for Medical Research and Occupational Health, Zagreb, Ksaverska c 2, Croatia. Fax: +385 14673303.
E-mail address: afucic@imi.hr (A. Fucic).

http://dx.doi.org/10.1016/j.mrfmmm.2015.12.003
0027-5107/

Radon levels, as a source of high LET radiation are increased in cases of naturally high radon background levels due to soil composition or as a consequence of surface mining which caused the exposure of layers with higher radon levels. Residential radon exposure is associated with higher lung cancer incidence in the general population, with an excess relative risk of 10% per 100 Bqm^3 [24–29]. Lung cancer incidence is increased among non-smokers [30], however additionally, the synergism between radon exposure and smoking has been suggested, as exposure to radon is particularly hazardous for smokers and recent ex-smokers [25,27,31].

At the present, the origin and possible biological or clinical significance of RCs have still not been elucidated [32]. The main difficulty is the lack of a causal association between exposures to xenobiotics and RC presence in human populations. Due to the fact that these cells are rare: 1–10–30 thousands of analyzed first division metaphases [11], a cytogenetic analysis in large cohorts is of great significance for the investigation of their frequency and potency as a biomarker of exposure and/or health risk.

This report of a long-term follow up study presents the RC frequency in peripheral blood lymphocytes in 3242 subjects of the general population, occupationally exposed subjects, lung cancer patients and children environmentally exposed to radon from the Kemerovo Region (south of Western Siberia, Russian Federation).

2. Materials and methods

2.1. Group characteristics

Cytogenetic monitoring was performed from 1985 to 2015 (30 years) as part of several different national projects on biomonitoring performed at Kemerovo State University, (Kemerovo, Russia). During this period, the analysis of chromosomal aberrations (CAs) was performed in 3242 residents of 22 settlements of the Kemerovo Region, divided into 5 main groups. The description of studied groups (number of individuals, number of scored cells, sex and age) is presented in Table 1. The recruitment of children and subjects from general public was implemented through national biomonitoring projects and oncological patients were analysed for purposes of projects in collaboration with clinics.

All subjects, or in case of children their parents, signed written consent and where informed about the aim of the study. The study was approved by Ethical committee of Kemerovo State University.

2.2. Urban residents

The group consisted of 734 donors living in 8 cities of the Kemerovo Region: Kemerovo, Novokuznetsk, Myski, Osinniki, Salair, Anzhero-Sudzhensk, Mezhdurechensk, Belovo. Kemerovo is the center of the chemical industry, Novokuznetsk of the metallurgical industry, while the others are focused mainly on the coal-mining industry. Almost 50% of this group were children and adolescents of school age (the average age was 24.3 years of age). The cytogenetic monitoring was performed from 1985 to 2012.

2.3. Factory workers

Workers in this study were biomonitored within the national occupational health surveillance program. Subjects were selected among the main industries represented in the Kemerovo region. All of the workers performing core manufacturing operations. In the study 832 industrial workers occupationally exposed to a complex of chemical and radiation factors: aluminum plant ($n = 80$), coke plant ($n = 139$), lead–zinc ore mining and processing plant ($n = 61$), coal mines ($n = 108$), heat power plant ($n = 444$). The mean age in this group was 42.8 years old (19–73 years old). The cytogenetic monitoring was performed from 1986 to 2014.

2.4. Rural population

512 donors living in 11 villages of the Kemerovo region. All of the villages were located away from industrial factories, so residents were not exposed to chemical contaminants. Almost 80% are children and adolescents of school age (the average age was 20.3 years). The cytogenetic monitoring was performed from 1994 to 2014.

2.5. Lung cancer patients

635 subjects living in the Kemerovo Region: 542 men and 93 women aged from 30 to 78 years old. The cytogenetic monitoring was performed from 2009 to 2014. Clinical and pathological diagnosis of disease was obtained for each patient. In study were included patients suffering from squamous cell non-keratinous and keratinous carcinomas, large cell carcinoma, small cell carcinoma, adenocarcinoma, differentiated carcinomas and sarcomas, undifferentiated carcinoma. Collection of blood samples for cytogenetic analysis in this group was held prior to diagnosis and before treatment.

2.6. Subjects with residential exposure to radon

529 children and adolescents, long-term resident in a boarding school (Tashtagol town, Kemerovo Region). This area is characterized by a woody mountain landscape with low levels of air pollution from chemical agents. However, multiple measurements (2007–2011) of radon volume activities carried out in the rooms of the boarding school showed an excess of critical radon concentrations (>200 Bq/m^3). The average volume radon activity in the residential areas of the boarding school was 468 ± 77 Bq/m^3 during all of the investigated years. The individual effective dose inhalation exposure due to isotopes of radon and its short-lived decay products was ~27 mSv/year [33]. The radiological monitoring was performed from 2007 to 2011. The cytogenetic monitoring was performed from 1992 to 2011.

2.7. Cell culture and aberration analysis

The peripheral blood lymphocytes were cultivated with phytohemagglutinin for 48 h using a conventional technique [32]. The whole blood obtained from the ulnar vein was cultivated. Volumes of 0.5 ml blood, 0.1 ml phytohaemagglutinin (PanEco, Russia), 6 ml RPMI-1640 (PanEco) and 1.5 ml calf serum were added to a culture flask. The duration of the cultivation was 48 h. Colchicine at a final concentration of 0.5 μg/ml was added to the culture, and the flasks were placed in an incubator for 2 h. At the end of the cultivation cycle, the preparations were centrifuged for 10 min at 1000 rpm, the supernatant was removed, and the pellet was resuspended. The pellets were placed in a hypotonic solution of 0.55% KCI for 10–15 min at 37 °C. The fixation of the material was performed in cooled fresh Carnoy's fixative (methanol and acetic acid in the ratio 3:1). The cell suspension was pipetted onto clean, cooled slides moistened with water. The preparations were encoded and stained with 2% Giemsa solution.

On average, 166 metaphases (100–2400) were scored for each individual. Scoring of CAs was performed blinded and without karyotyping. All kinds of intra- and interchromatid exchanges, single fragments were registered as chromatid-type aberrations. Polycentric- and centric rings, acentric rings, double fragments, and abnormal monocentrics were registered as chromosome-type