Mutation Research 784 (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% KCl 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

Table 1
Structure and characteristics of the studied groups.

No	Study group	No of individuals	No of examined cells	Mean age (years) ± SD	Range (years)	Smokers %
1	Urban residents:					
	Men	427	59212	29.3 ± 7.0	8–63	33.5
	Women	307	32171	17.1 ± 7.1	8–53	14.3
	Total	734	91383	24.3 ± 15.0	8–63	25.5
2	Factory workers:					
	Men	553	67382	42.9 ± 10.9	19–73	60.4
	Women	279	27900	42.5 ± 8.4	21–59	15.8
	Total	832	95282	42.8 ± 13.4	19–73	45.4
3	Rural population:					
	Men	202	31643	20.2 ± 13.6	5–71	20.3
	Women	310	47379	20.4 ± 13.2	4–78	11.0
	Total	512	79022	20.3 ± 13.4	4–78	14.7
4	Lung cancer patients:					
	Men	542	102970	60.0 ± 7.5	31–78	91.3
	Women	93	14492	58.0 ± 8.2	30–74	28.0
	Total	635	117462	59.7 ± 7.6	30–78	82.1
5	Children and adolescents with residential exposure to radon:					
	Men	280	87160	13.5 ± 2.7	7–23	
	Women	249	72975	13.5 ± 2.6	8–19	
	Total	529	160135	13.5 ± 2.6	7–23	24%[a]
	TOTAL	3242	543269			

[a] In group of older adolescents (<16 years).

aberrations. Gaps were not included into the number of CAs and were scored separately.

All analyses were performed by two scorers. Each rogue cell was confirmed by two scorers. Rogue cells were documented by photograhy.

2.8. *Definition of rogue cells*

There is still no strong definition of RCs [32], but the presence of double minutes has been indicated as a characteristic feature of these cells [1]. The presence of multiple chromosome-type exchanges (polycentric, ring chromosome, translocation or inversion) seems to be a commonly accepted feature. In the present work, an RC was defined as a cell having at least 3 chromosome-type exchanges and "excess" acentrics as double fragments and (or) double minutes. RC chromosome aberrations were not included in the final score of genome damage per subject.

2.9. *Radon measurement*

The radon level was measured in homes and classrooms of a boarding school in town Tashtagol, as well as in selected villages in the period 2007–2011. Measurements of the volume of radon activity in the air were performed using the radon radiometer PPA-01M-01 (Alfarad) ("Zashita", Ltd., Moscow, Russia) in the Air 1 mode, which includes air sampling by a built-in blower for 3 min and analysis of the sample with the subsequent show of the integral value for 20 min. USK "Progress" (Yekaterinburg, Russia) with a set of carbon absorbers was used for the integrated assessment of radon concentration. The integral method is based on the exposure of the absorbers with activated charcoal for 1 day and then *in vitro* measuring of the activity of the radon adsorbed in the activated carbon.

The individual effective dose was calculated by the following formula:

$$E = \Sigma d_i \times m_i \times C_i, \text{ mSv/year}$$

(d_i—dose coefficient for the *i* radionuclide in its pre-oral intake with foods; C_j—the average specific activity of the *j* radionuclide in the *i* component of the diet, Bq/kg; m_i—the average annual consumption of the *i* product, kg/year).

All measurements of radon levels were performed indoor during winter (January – February) which eliminated possible bias caused by the difference in temperature or humidity [34]. Applied method according to recent studies did not show a significant difference in reliability in comparison with other methods for radon measurements [35,36].

2.10. *Statistical analysis*

The results were processed statistically using the STATISTICA software package (Statsoft, United States). The validity of inter group differences was estimated by the Mann–Whitney U-test. Differences were considered significant at the confidence level $p < 0.05$.

3. Results

As a result of the analysis of 543269 metaphases obtained from 3242 donors for the period from 1986 to 2014, we found 54 cells had a typical roque phenotype. Thus, the frequency of RCs in the total sample of Kemerovo Region population was 0.1 per 1000 cells. A detailed description of these cells, including their ranking according to the number of chromosome-type exchanges, as well as the presence or absence in them of free double fragments and, separately, double minutes, is presented in Table 2. Example of rogue cell is given in Fig. 1.

The minimum number (3) chromosome-type exchanges, as noted in section "Definition of rogue cells", was observed in 9 of 54 RCs (16,6%), in this case in each of the said cells double minutes and/or double free fragments were observed additionally. More than 50% (51.9) of the total RCs had 6 or more chromosome-type exchanges. The maximum number of exchanges registered in one cell was 21: tric - 6, dic - 7, t - 2, fra > 10, dm > 10.

The number of subjects who were carriers of RCs significantly differed between the examined groups. Only 1 donor among the 734 urban residents from group 1 had RCs (0.01 ± 0.003 per 1000 cells). In group 2 (factory workers), only 2 men (aluminum plant workers and coke plant workers) had 1 RC each. Frequency of RCs

Table 2
Characteristics of RCs in all follow-up subjects from the Kemerovo region.

Number of chromosome-type exchanges per RCs[a]	Chromosome-type exchanges, N (%)	Double fragments,yes/no	Double minutes,yes/no
3	9 (16,6)	9/0	6/3
4	10 (18,5)	8/2	7/3
5	7 (13,0)	6/1	6/1
6 or more	28 (51,9)	27/1	27/1
Total RCs	54 (100)	50/4	46/8

[a] Polycentric chromosomes were expressed as dicentric equivalents, where dicentric equivalents equaled the number of centromeres minus one.

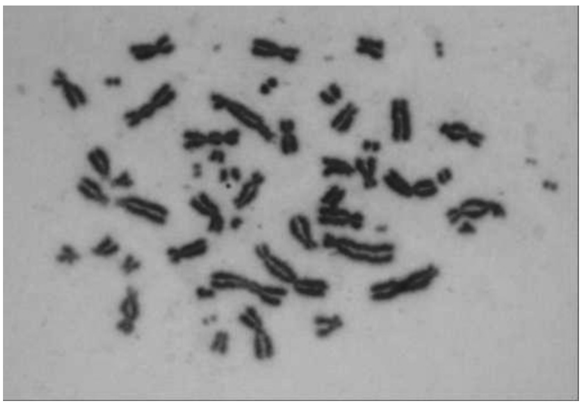

Fig. 1. Metaphase of rogue lymphocyte.

in group 2 was 0.02 ± 0.008 per 1000 cells and was no different from the same indicator in group 1 (0.588; Mann–Whitney U-test). In group 3 (512 villagers), we found 5 teenagers each having 1 RC. All of these donors lived in only 2 of the 11 villages included in the monitoring. We discovered an increase of the RC frequency in this group (0.06 ± 0.005 per 1000 cells) in comparison with group 1 and group 2, but this tendency was not significant ($p = 0.069$ and $p = 0.166$, respectively). Typical RCs were diagnosed in 11 cases of 117462 metaphases scored from lung cancer patients. All of these patients were men aged from 55 to 62, all smokers. The spectrum of the pathomorphological types of lung cancer in this group was as follows: squamous cell carcinoma – 8 cases, adenocarcinoma – 2 cases, large cell carcinoma – 1 case. Therefore, the RC frequency in group 4 (0.09 ± 0.015 per 1000 cells) was higher in comparison with groups 1 and 2 ($p < 0.05$), but differences between group 4 and 3 were not significant ($p = 0.464$).

RC “carriers” were the most abundant (6.2%) among children and adolescents with residential exposure to radon. In this group, 31 individuals had 1 RC each, while two cases reported approx. 2 RCs. The RC frequency in this group was 0.22 ± 0.01 per 1000 cells. This value was significantly higher in comparison with the other observed groups.

Frequency of RCs during the follow-up period in the cohort of children living in a boarding school and radon levels in the boarding school (1992–2011) is shown in Table 3.

During the entire follow up period, the lymphocytes of children and adolescents had RCs (the frequency of RCs varied from 0.14 per 1000 cells in 2005 to 0.49 per 1000 cells in 2011). Sex did not influence the RC frequency in exposed children: 19 male (57.6%) and 14 female (42.4%) had such cells. Age, duration of residents and smoking did not influence RC frequency in conditions of radon exposure.

Of particular interest was the comparison between the occurrence of RCs and the frequency of “simple” chromosomal damage in the non-rogue cells of the same individual [7]. The results of this analysis for the two-subject category where it the maximum number of carrier RCs was found—subjects with residential exposure to radon and lung cancer patients are presented in Table 4.

The mean value of “simple” chromosomal damage frequency in the non-rogue cells in group of subjects with residential exposure to radon having RCs (4.77 ± 2.13) and the frequency of chromosome-type exchanges per 100 metaphases (0.23 ± 0.54) did not differ from subjects without RCs (4.4 ± 2.47 and 0.21 ± 0.41, respectively). At the same time, the group of lung cancer patients was characterized by a significantly higher level of CAs per 100 metaphases (4.44 ± 1.62) and chromosome-type exchanges per 100 metaphases (0.34 ± 0.32) in donors with RCs.

4. Discussion

This long-term cross sectional follow-up study of cytogenetic damages in the blood lymphocytes of residents from the Kemerovo Region (Siberia, Russian Federation) showed that the RC frequency in this population was 1 per 10000 metaphases. This frequency is within levels reported by other studies in various cohorts [1,5,11]. The present study shows significant difference in RC frequencies between the general population, occupationally exposed subjects, lung cancer patients and children residentially exposed to radon. The highest RC frequency (0.22 ± 0.01 per 1000 metaphases) was discovered in the group of residents of areas with radon levels over 200 Bq/m^3 during the whole period of follow up from 1992 to 2011 (Table 3). Although the RC frequencies did not correlate with radon levels, it could be suggested that radon levels cause multiple genome damages when compared with the other investigated groups. In our previous studies, we showed that children from this group are characterized by a significant increase in genome damage in lymphocytes [37,38] and exfoliated cells [39]. In these studies, we found no significant anthropogenic factors capable of modifying the frequency of cytogenetic damage, such as the action of environmental pollution or morbidity, sex and age. For the purpose of this study, investigation of air and water samples for the estimation of environmental pollution in the boarding school territory was performed. Probes for the Ames test showed no mutagenic effects on cultures of *Salmonella typhimurium* TA98 and TA100. Finally, we discovered no mutagenic activity of air and water samples [33].

In this study, 85% of the scored RCs contained double minutes (DMs), gene amplifications frequently seen in cancer cells, which contribute to carcinogenesis and have been suggested as a supplement of cancer diagnosis [40]. Thus, as DMs are related with several types of solid tumors including lung cancer [41], additional significance could be suggested for RCs as a biomarker. The importance of DMs in cancer biology may be seen in the fact that their elimination has recently been introduced as a part of chemotherapy [42]. As DMs are present in complex genome rearrangements of lung cancer cell lines, it is possible that a single catastrophic event model is involved in the formation of the amplicons, including the MYCN locus, and related with lung cancer promotion [43,44]. Additionally, in clones derived from cells exposed to alpha-particles, cells with extensive chromosome breaking and double minutes have been reported [45].

Taking into account the results of previous investigations, which showed a possible influence of contagious factors on RC origin [4,7], we noted certain acute respiratory diseases in children at the

Table 3
Frequency of RCs in children and adolescents with residential exposure to radon in different years of the study.

Year of study	No of person	No of cells	No of RCs	RCs per 1000 cells	Average radon volume activity[a]	
Bq/m^3, mean ± standard error	Range, Bq/m^3					
1992	28	2800	1	0.36 ± 0.02	no data	–
2005	196	42639	6	0.14 ± 0.01	no data	–
2007	66	12064	2	0.17 ± 0.01	235 ± 44	68–583
2009	79	66522	12	0.33 ± 0.01	441 ± 88	110–1373
2010	68	13600	3	0.22 ± 0.01	905 ± 134	680–1143
2011	92	22510	11	0.49 ± 0.02[b]	347 ± 101	74–749

[a] The radon volume activity in buildings of the boarding school.
[b] Significant difference in comparison with 2005 and 2009 ($p < 0.02$).

Table 4
The occurrence of simple chromosomal damages in lymphocytes, in relation to the detection of the RCs.

Subject category	Subjects with residential exposure to radon		Lung cancer patients	
RC +	RC −	RC +	RC −	
No of person	33	496	11	625
CA per 100 metaphases	4.77 ± 2.13	4.4 ± 2.47	4.44 ± 1.62*	3.1 ± 2.1
Chromosome-type exchanges per 100 metaphases	0.23 ± 0.54	0.21 ± 0.41	0.34 ± 0.32**	0.2 ± 0.4

moment of blood collection or for the period of 3 months before it. An analysis of questionnaires and individual medical cards showed that only 2 of the 33 children with RCs were characterized by an increased level of a respiratory disease. Due to the biological characteristics of the study area (mountain taiga area), the possibility of infection with tick-borne encephalitis virus children, especially in spring and summer was also considered. An estimation of the potential of this contagious factor to contribute to RC origin showed that, despite the rather high percentage of tick bites in the total sample, children who had a metaphase with multiple aberrations were not bitten by ticks for at least one year before the examination.

A number of studies have demonstrated the genotoxic risk, including clastogenic, of radon exposure. Uranium miners or other miners exposed to high doses of radon emission are characterized by a higher level of cytogenetic damage in their peripheral blood lymphocytes compared to control groups [46–52]. Environmental radon exposure may induce micronuclei formation, and both stable and unstable chromosomal aberrations in lymphocytes [53,55]. Rogue cells were in such studies reported only anecdotally. Researchers from Slovenia [56] performed investigations of CA and micronuclei (MN) frequency in blood lymphocytes in pupils of elementary school burdened by high concentrations of radon in classrooms. In the winter season, the radon concentration in the classrooms was 7.000 Bq/m^3. Yearly effective doses for pupils, estimated according to ICRP 65, ranged from 7 to 11 mSv. There are some significant differences in cytogenetic changes between the exposed and control groups of pupils. Altogether 85 pupils (37 girls and 48 boys) from the first four grades between the ages of 9 and 12 years old were examined and control pupils were sampled from a school where radon concentration did not exceed 400 Bq/m^3. CA frequency in the exposed group (2.03 ± 0.87) was increased in comparison with controls (1.21 ± 0.59). However, they found no rogue-phenotype of 62861 observed metaphases.

In addition, German researchers [54] published results of investigation of chromosomal aberrations in the lymphocytes of 25 donors, living for a longer period of time in houses with indoor radon concentrations exceeding 4–60-fold the German average of 50 Bq/m^{-3}. Besides the significant increase of the frequency of dicentric and ring chromosomes, subjects exposed to radon had 5 cells with “multiple chromosomal damage”; 2 of 5 such cells referred to typical RCs (several dicentric chromosomes + acentrics).

A study in which 2346 persons (Chernobyl liquidators, personnel of radiochemical plants, atomic research and industry personnel, uranium miners, cosmonauts, as well as residents of contaminated and non-contaminated with radionuclides territories) were investigated for CA showed that among 936 273 metaphases, 98 RCs (multiaberrant cells) (0.11 per 1000 cells) were found. The highest frequency of RCs (2.49 per 1000 cells) was discovered in a group of radiochemical plant personnel that had contact with plutonium salts. In a group of uranium miners with radon exposure from Tselinograd (Kazakhstan), RCs were second according relevance; 0.37 per 1000 cells. The analysis of RC presence in various cohorts suggests that an origin of multiaberrant metaphases is a result of internal exposure to high LET radiation from alpha-particles [22–24,37], which is in concordance with our results, as alpha-particles are the predominant form of radiation emitted during radon decay [57]. α-particles have high LET radiation (110–180 keV/μm). Statistically, the most likely target of ionization would in living system is a molecule H_2O (radiolysis of water) resulting in a large amount of oxygen radical species capable of destroying the structure of macromolecules. In addition to the primary ionization along the track of the particle motion numerous foci of secondary ionization are produced near the axis of motion of the particle. Ionization field in the span of α-particles is therefore a cylinder, the axis of which has a powerful primary ionization, and on the periphery numerous secondary ionization events. If on the path of a particle stands a DNA molecule there would be a high degree of probability that both DNA strands are broken. Studies have show that the local radiation load in the case of α-particles is extremely high and can reach 10–50 cGy. Clearly the introduction of such radiation energy in a small volume leads to the formation of large clusters of multiple ionization and the local formation of numerous breaks in the DNA structure [57,58]. Such mechanism of action of α-particles is shown in experimental results from *in vitro* studies in which blood was irradiated by radon in the range dose between 0 and 127 mGy which caused formation of multiaberrant genome including double minutes [59].

The current study shows that the next group with the highest frequency of RC after children residentially exposed to radon was group of lung cancer patients (0.09 ± 0.015 per 1000 cells). Earlier Russian studies reported RCs in peripheral lymphocytes of Hodgkin’s lymphoma patients after chemotherapy [60]. However in our study, lung cancer patients were sampled before chemotherapy and radiotherapy. It was recently show that lung cancer patients are prone to genome instability due to decreased DNA repair capacity [61] and that this phenomenon is measurable in lymphocytes [62]. We can hypothesize that the RC carriers in the lung cancer patients were exposed to radon the same as in children from the current study with measured increased radon levels. Consequently, it could be suspected that 11 lung cancer patients having RCs could be radiosensitive and exposed to radon as a well described risk of lung cancer development [31,62–64]. The future investigation will include an analysis of radon levels in the region of residence of the lung cancer patients.

Difference in interindividual radiosensitivity is probably the reason why in all children exposed to indoor radon RCs were not detected. Another reason may be in the different number of analyzed cells per person as RCs are a rare event. There is a difference in type of genome damage between children and lung cancer patients. Thus in the lymphocytes of lung cancer patients who had RCs, significantly more metaphases with single aberrations were detected, including chromosome-type exchanges. On the other hand, in a cohort of pupils of the boarding school exposed to radon, this pattern was not observed (Table 4). Assessing this difference, it should be stressed that other differences in environmental settings between lung cancer patients and pupils also exist.

Radiological investigations of rural regions where 5 subjects with RCs were detected showed an increased concentration of radon in some houses (average radon volume activity: from 368 to 534 Bq/m^3). At least 2 of the 5 RCs carriers were exposed to significantly higher levels of radon than recommended by the WHO [29]. The lowest RC frequency (0.01 ± 0.003 per 1000 cells and 0.02 ± 0.008 per 1000 cells) was observed in groups 1 and 2. In the first case, the carrier of RCs was a healthy boy (14 years old) living in a non-industrial area of Kemerovo. Other cases are workers from the chemical industry. It should be pointed out that we performed no radiological investigations in the places of residence of these donors so we cannot exclude or confirm radon radiation in these cases.

5. Conclusion

In conclusion, the results of this study, the largest 30 years long follow up study of RC by CA analysis, suggest that radon and its decay daughter products (typical alpha-emitters) may be the leading factors causing RC in subjects exposed to high LET radiation. Thus, RC may be a candidate biomarker of exposure to radiation with high LET. Environmentally exposed children seem to be most susceptible population with the highest RC frequency. The possible synergistic mechanism between smoking and radon exposure on the frequency of RC formation needs further investigation.

Funding

State task (No.2162).

Conflict of interest

None declared.

Acknowledgements

We are particularly grateful to Prof. Vyacheslav S. Zurkov (Institute of Human Ecology and Environmental Hygiene A.N. Sysina, Moscow, Russian Federation) for his valuable advice; to Dr Lyudmila V. Ahaltseva (Institute of Human Ecology and Environmental Hygiene A.N. Sysina, Moscow, Russian Federation) for their help in performing of the Aims test; to Dr Natalia V. Sorokina (Kemerovo State University, Kemerovo, Russian Federation) for her help in performing radiological investigations.

References

[1] A.A. Awa, J.V. Neel, Cytogenetic "rogue" cells: what is their frequency, origin and evolutionary significance? Proc. Natl. Acad. Sci. U. S. A 83 (1986) 1021–1025.
[2] A.D. Bloom, J.V. Neel, K.W. Choi, S. Iida, N. Chagnon, Chromosome aberrations among the Yanomamma Indians, Proc. Natl. Acad. Sci. U. S. A 66 (1970) 920–927.
[3] A.D. Bloom, J.V. Neel, T. Tsuchimoto, K. Meilinger, Chromosome breakage in leukocytes of South American Indians, Cytogenet. Cell Genet. 12 (1973) 175–186.
[4] J.R. Lazutka, J.V. Neel, E.O. Major, V. Dedonyte, J. Mierauskine, G. Slapsyte, A. Kesminiene, High titers of antibodies to two human polyomaviruses, JCV and BKV, correlate with increased frequency of chromosomal damage in human lymphocytes, Cancer Lett. 109 (1996) 177–183.
[5] J.V. Neel, A.A. Awa, Y. Kodama, M. Nakano, K. Mabuchi, "Rogue" lymphocytes among Ukrainians not exposed to radioactive fall-out from the Chernobyl accident: the possible role of this phenomenon in oncogenesis teratogenesis, and mutagenesis, Proc. Nat. Acad. Sci. U. S. A 89 (1992) 6973–6977.
[6] J.V. Neel, E.O. Major, A.A. Awa, T. Glover, A. Burgess, R. Traub, B. Curfman, C. Satoh, Hypothesis: Rogue cell-type chromosomal damage in lymphocytes is associated with infection with the JC human polyoma virus and has implications for oncogenesis, Proc. Nat. Acad. Sci. U. S. A 93 (1996) 2690–2695.
[7] J.V. Neel, An association, in adult Japanese, between the occurrence of rogue cells among cultured lymphocytes (JC virus activity) and the frequency of "simple" chromosomal damage among the lymphocytes of persons exhibiting these rogue cells, Am. J. Hum. Genet. 69 (1998) 489–497.
[8] Y.R. Ahuja, G. Obe, Are rogue cells an indicator of cancer risk due to the action of bacterial restriction endonucleases? Mutat. Res. 310 (1994) 103–112.
[9] E.J. Tawn, C.L. Cartmel, E.M.T. Pyta, Cells with multiple chromosome aberrations in control individuals, Mutat. Res. 144 (1985) 247–250.
[10] L. Verschaeve, E.V. Domracheva, S.A. Kuzuetsov, V.V. Nechai, Chromosome aberrations in inhabitants of Byelorussia: consequence of the Chernobyl accident, Mutat. Res. 287 (1993) 253–259.
[11] N.P. Bochkov, L.D. Katosova, Analysis of multiaberrant cells in lymphocytes of persons living in different ecological regions, Mutat. Res. 323 (1994) 7–10.
[12] R. Mustonen, C. Lindholm, E.J. Tawn, L. Sabatier, S. Salomaa, The incidence of cytogenetically abnormal rogue cells in peripheral blood, Int. J. Radiat. Biol. 74 (1998) 781–785.
[13] V.D. Cheriyan, C.J. Kurien, B. Das, E.N. Ramachandran, C.V. Karuppasamy, M.V. Thampi, K.P. George, P.C. Kesavan, P.K. Koya, P.S. Chauhan, Genetic monitoring of the human population from high-level natural radiation areas of Kerala on the southwest coast of India.II.Incidence of numerical and structural chromosomal aberrations in the lymphocytes of newborns, Radiat. Res. 152 (1999) 154–158.
[14] M.A. Pilinskaya, A.M. Shemutun, S.S. Dybski, D.V. Redko, I.A. Znaevskaya, Identification of the multiaberrant lymphocytes at cytogenetic examination of different groups of persons who have contact with mutaganiec factors, Tsitotol. Genet. 28 (1) (1994) 27–32.
[15] A.V. Sevankaev, A.F. Tsyb, D.C. Lloyd, A.A. Zhloba, V.V. Moiseenko, A.M. Skrjabin, V.M. Climov, "Rogue" cells observed in children exposed to radiation from the Chernobyl accident, Int. J. Radiat. Biol. 63 (1993) 361–367.
[16] G. Obe, I. Johannes, C. Johannes, K. Hallman, G. Reitz, R. Facius, Chromosomal aberrations in blood lymphocytes of astronauts after long-term space flights, Int. J. Radiat. Biol. 72 (1997) 727–734.
[17] S. Salomaa, A.V. Sevankaev, A.A. Zhloba, E. Kumpusalo, S. Mackinen, C. Lindholm, L. Kumpusalo, et al., Unstable and stable chromosome aberrations in lymphocytes of people exposed to Chernobyl fallout in Bryansk, Russia, Int. J. Radiat. Biol. 71 (1997) 51–59.
[18] E.V. Domracheva, N.B. Rivkind, E.A. Aseeva, T.N. Obukhova, L.V. D'achenko, A.I. Vorobiov, Stable and unstable aberrations in lymphocytes of Chernobyl accident clearance workers carrying rogue cells, Appl. Radiat. Isot. 52 (2000) 1153–1159.
[19] R. Rozgaj, V. Kasuba, D. Simic, The frequency of dicentrics and acentrics and the incidence of rogue cells in radiation workers, Mutagenesis 17 (2002) 135–139.
[20] K. Tanaka, S. Iida, N. Takeichi, N.J. Chaizhunusova, B.I. Gusev, K.N. Apsalikov, T. Inaba, M. Hoshi, Unstable-type chromosome aberrations in lymphocytes from individuals living near Semipalatinsk nuclear test site, J. Radiat. Res. 47 (Suppl. A) (2006) 159–164.
[21] E.V. Domracheva, S.A. Kuznetsov, N.E. Shklovski-Kordi, A.I. Vorobiov, Cells with multiple chromosomal aberrations in residents of Chernobyl area (in Russian), Gematol. Transfuziol. 11 (1991) 36–37.
[22] N.A. Popova, L.P. Nazarenko, S.A. Nazarenko, Multiaberrant cell formation caused by exposure to internal densely-ionizing irradiation, Russ. J. Genet. 40 (2004) 1419–1422.
[23] E.A. Aseeva, G.P. Snigireva, A.L. Neverova, N.N. Novitskaia, E.D. Khazins, E.V. Domracheva, Multiaberrant cells in groups of people exposed to radiation in different situations and their possible biological role, Biophysics 55 (2010) 496–503.

[24] J.M. Barros-Dios, M.A. Barriero, A. Ruano-Ravina, A. Figueiras, Exposure to residential radon and lung cancer in Spain: a populaton-based case-control study, Am. J. Epidemiol. 156 (2002) 548–560.
[25] O. Catelinois, A. Rogel, D. Laurier, S. Billon, D. Hemon, P. Verger, M. Tirmarche, Lung cancer attributed to indoor radon exposure in France: impact of the risk models and uncertainty analysis, Environ. Health Perspect. 114 (2006) 1361–1366.
[26] L. Kreienbrock, M. Kreuzer, M. Gerken, G. Dingerkus, J. Wellmann, G. Keller, D. Krewski, J.H. Lubin, J.M. Zielinski, M. Alavanja, V.S. Catalan, R.V. Field, J.B. Klotz, E.G. Letourneau, C.F. Lynch, J.I. Lyon, D.P. Sandler, J.B. Schoenberg, D.J. Steck, J.A. Stolwijk, C. Weinberg, H.B. Wilcox, Residential radon and risk of lung cancer: a combined analysis of 7 North American case-control studies, Epidemiology 16 (2005) 137–145.
[27] G. Lagarde, L. Axelsson, H. Damber, F. Mellander, Residential radon and lung cancer among never-smokersin Sweden, Epidemiology 12 (2001) 396–404.
[28] G. Pershagen, G. Akerblom, O. Axelson, B. Clavensjo, L. Damber, G. Desai, A. Enflo, F. Lagarde, H. Mellander, M. Svartengren, G. Swedjemark, Residential radon exposure and lung cancer in Sweden, New Eng. J. Med. 330 (1994) 59–164.
[29] World Health Organization Handbook on indoor radon: a public health perspective, Ed. H. Zeeb, F. Shannoun 2009.
[30] M. Torres-Duran, A. Ruano-Ravina, I. Parente-Lamelas, V. Leiro-Fernández, J. Abal-Arca, C. Montero-Martinez, C. Pena-Alvarez, F.J. Gonzalez-Barcala, O. Castro-Anon, A. Golpe-Gomez, C. Martinez, M.J. Mejuto-Marti, A. Fernandez-Villar, J.M. Barros-Dios, Eur. Respir. J. 44 (4) (2014) 994–1001.
[31] S. Darby, D. Hill, A. Auvinen, J.M. Barros-Dios, H. Baysson, F. Bochicchio, F. Deo, R. Falk, F. Forastiere, M. Hakama, I. Heid, L. Kreienbrock, M. Kreuzer, F. Lagarde, I. Makelainen, C. Muirhead, W. Oberaigner, G. Pershagen, A. Ruano-Ravina, E. Ruosteenoja, A.S. Rosari, M. Tirmarche, L. Tomasek, E. Whitley, H.E. Wichmann, R. Doll, Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 case-control studies, B.M.J. 330 (7485) (2005) 223.
[32] International Atomic Energy Agency, Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies, IAEA, VIENNA IAEA-EPR (2011) 29–31.
[33] V.G. Druzhinin, N.L. Aluker, L.V. Akhal'tseva, T.A. Golovina, F.I. Ingel, A.V. Larionov, N.V. Sorokina, T.A. Tolochko, A.V. Shaposhnikova, N.A. Iurtseva, Toxic and genetic risk assessments in children of Gornaya Shoria (in Russian), Gig. Sanit. 3 (2010) 12–18.
[34] A. Cooper, T.N. Le, T. Iimoto, T. Kosako, Temperature calibration formula for activated charcoal radon collectors, J. Environ. Radioact. (2011) 60–63.
[35] T.R. Beck, E. Foerster, H. Buchroder, V. Schmidt, J. Doring, The measurement accuracy of passive radon instruments, Radiat. Prot. Dosimetry 158 (1) (2014) 59–67.
[36] M. Janik, T. Ishikawa, Y. Omori, N. Kavasi, Invited article: radon and thoron intercomparison experiments for integrated monitors at NIRS, Japan, Rev. Sci. Instrum. 85 (2) (2014) 022001.
[37] M.Y. Sinitsky, V.G. Druzhinin, The application of the cytokinesis block micronucleus assay on peripheral blood lymphocytes for the assessment of genome damage in long-term residents of areas with high radon concentration, J. Radiat. Res. 55 (2014) 61–66.
[38] V.G. Druzhinin, M. Yu. Sinitsky, A.V. Larionov, V.P. Volobaev, V.I. Minina, T.A. Golovina, Assessing the level of chromosome aberrations in peripheral blood lymphocytes in long-term resident children under conditions of high exposure to radon and its decay products, Mutagenesis 30 (5) (2015) 677–683.
[39] A.V. Meier, V.G. Druzhinin, A.V. Larionov, T.A. Tolochko, Genotoxic and cytotoxic effects in buccal cells of children living in ecologically different Kuzbass areas (in Russian), Tsitologiia 52 (2010) 305–310.
[40] M. Hayes, J. Li, An integrative framework for the identification of double minute chromosomes using next generation sequencing data, BMC Genet. 16 (Suppl. 2) (2015) S1.
[41] J.L. Nielsen, J.T. Walsh, D.R. Degen, S.M. Drabek, J.R. McGill, D.D. von Hoff, Evidence of gene amplification in the form of double minute chromosomes is frequently observed in lung cancer, Cancer Genet. Cytogenet. 65 (2) (1993) 120–124.
[42] L. Yu, Y. Zhao, C. Quan, W. Ji, J. Zhu, Y. Huang, R. Guan, D. Sun, Y. Jin, X. Meng, C. Zhang, Y. Yu, J. Bai, W. Sun, S. Fu, Gemcitabine eliminates double minute chromosomes from human ovarian cancer cells, PLoS One 8 (8) (2013), e71988.
[43] V. Pistoia, F. Morandi, A. Pezzolo, L. Raffaghello, I. Prigione, MYCN: from oncoprotein to tumor-associated antigen, Front. Oncol. 2 (2012) 174.
[44] K. Kitada, S. Aida, S. Aikawa, Coamplification of multiple regions of chromosome 2, including MYCN, in a single patchwork amplicon in cancer cell lines, Cytogenet. Genome Res. 136 (1) (2012) 30–37.
[45] M. Durante, G.F. Grossi, T.C. Yang, Radiation-induced chromosomal instability in human mammary epithelial cells, Adv. Space Res. 18 (1-2) (1996) 99–108.
[46] W. Popp, U. Plappert, W.U. Mũller, et al., Biomarkers of genetic damage and inflammation in blood and bronchoalveolar lavage fluid among former German uranium miners: a pilot study, Radiat. Environ. Biophysics 39 (2000) 275–282.
[47] Z. Smerhovsky, K. Landa, P. Rössner, D. Juzova, M. Brabec, Z. Zudova, N. Hola, H. Zarska, E. Nevsimalova, Increased risk of cancer in radon-exposed miners with elevated frequency of chromosomal aberrations, Mutat. Res. 514 (2002) 165–176.
[48] R.J. Srám, P. Rössner, Z. Smerhovský, Cytogenetic analysis and occupational health in the Czech Republic, Mutat. Res. 566 (2004) 21–48.
[49] G. Mészáros, G. Bognár, G.J. Köteles, Long-term persistence of chromosome aberrations in uranium miners, J. Occup. Health 46 (2004) 310–315.
[50] R. Zaire, M. Notter, W. Riedel, E. Thiel, Unexpected rates of chromosomal instabilities and alterations of hormone levels in Namibian uranium miners, Radiat. Res. 147 (1997) 579–584.
[51] D.C. Lloyd, J.N. Lucas, A.A. Edwards, et al., A study to verify a reported excess of chromosomal aberrations in blood lymphocytes of Namibian uranium miners, Radiat. Res. 155 (2001) 809–817.
[52] G. Wolf, D. Arndt, N. Kotschy-Lang, G. Obe, Chromosomal aberrations in uranium and coal miners, Int. J. Radiat. Biol. 80 (2004) 147–153.
[53] M. Bilban, C.B. Jakopin, Incidence of cytogenetic damage in lead–zinc mine workers exposed to radon, Mutagenesis 20 (2005) 187–191.
[54] M. Bauchinger, E. Schmid, H. Braselmann, U. Kalka, Chromosome aberrations in peripheral lymphocytes from occupants of houses with elevated indoor radon concentrations, Mutat. Res. 310 (1994) 135–142.
[55] C. Lindholm, I. Mäkeläinen, W. Paile, A. Koivistoinen, S. Salomaa, Domestic radon exposure and the frequency of stable or unstable chromosomal aberrations in lymphocytes, Int. J. Radiat. Biol. 75 (1999) 921–928.
[56] M. Bilban, J. Vaupoti, Chromosome aberrations study of pupils in high radon level elementary school, Health Phys. 80 (2001) 157–163.
[57] National Research Council, Health Effects of Exposure to Radon (BEIR VI). (1999) National Academy Press Washington, DC.
[58] D.T. Goodhead, Initial Events in the cellular effects of ionizing radiations: clustered damage in DNA, Int. J. Rad. Biol. 65 (1) (1994) 7–17.
[59] V.Z. Hamza, M.N. Mohankumar, Cytogenetic damage in human blood lymphocytes exposed *in vitro* to radon, Mutat. Res. 661 (2009) 1–9.
[60] N.I. Ryabchenko, V.A. Nasonova, E.V. Fesenko, T.V. Kondrashova, M.M. Antoschina, V.V. Pavlov, N.V. Ryabikina, Aberrant and multiaberrant (rogue) cells in peripheral lymphocytes of Hodgkin's lymphoma patients after chemotherapy, Mutat. Res. 601 (2006) 61–70.
[61] Q. Wei, H. Liu, Z. Liu, C.I. Amos, J.A. Doherty, H. Bickeboller, R.J. Hung, P. Brennan, R. Houlston, M.T. Landi, N.E. Caporaso, D. Christiani, A novel variant in DNA repair gene GTF2H4 is associated with lung cancer risk: A reanalysis of GWAS datasets from the TRICL consortium, Cancer Res. 75 (15 Suppl) (2015), Abstract no. 4597.
[62] A. Rosenberger, U. Rössler, S. Hornhardt, W. Sauter, H. Bickeböller, H.E. Wichmann, M. Gomolka, Heritability of radiation response in lung cancer families, Genes (Basel) 3 (2) (2012) 248–260.
[63] A. Robertson, J. Allen, R. Laney, A. Curnow, The cellular and molecular carcinogenic effects of radon exposure: a review, Int. J. Mol. Sci. 14 (2013) 14024–14063.
[64] J.M. Samet, R.W. Hornung, Review of radon and lung cancer risk, Risk Anal. 10 (1990) 65–75.