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# Hypermethylation of gene promoters in peripheral blood leukocytes in humans long term after radiation exposure

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ABSTRACT

Some human genes known to undergo age-related promoter hypermethylation. These epigenetic modifications are similar to those occurring in the course of certain diseases, e.g. some types of cancer, which in turn may also associate with age. Given external genotoxic factors may additionally contribute to hypermethylation, this study was designed to analyzes, using methylation-sensitive polymerase chain reaction (PCR), the CpG island hypermethylation in *RASSF1A, CDKN2A* (including *p16/INK4A* and *p14/ARF*) and *GSTP1* promoters in peripheral blood leukocytes of individuals exposed to ionizing radiation long time ago. One hundred and twenty-four irradiated subjects (24–77 years old at sampling: 83 Chernobyl Nuclear Power Plant clean-up workers, 21 nuclear workers, 20 residents of territories with radioactive contamination) and 208 unirradiated volunteers (19–77 years old at sampling) were enrolled. In addition, 74 non-exposed offspring (2–51 years old at sampling) born to irradiated parents were examined. The frequency of individuals displaying promoter methylation of at least one gene in exposed group was significantly higher as compared to the control group (OR=5.44, 95% CI=2.62–11.76, $p=3.9\times10^{-7}$). No significant difference was found between the frequency of subjects with the revealed promoter methylation in the group of offspring born to irradiated parents and in the control group. The increase in the number of methylated loci of *RASSF1A and p14/ARF* was associated with age ($\beta=0.242$; $p=1.7\times10^{-5}$). In contrast, hypermethylation of *p16/INK4A* and *GSTP1* genes correlated with the fact of radiation exposure only ($\beta=0.290$; $p=1.7\times10^{-7}$). The latter finding demonstrates that methylation changes in blood leukocytes of healthy subjects exposed to radiation resemble those reported in human malignancies. Additional studies are required to identify the dose-response of epigenetic markers specifically associating with radiation-induced premature aging and/or with the development of age-associated cancer and non-cancer diseases.



## 1. Introduction

CpG island methylation is a major epigenetic modification playing a key role in a number of biological processes. This modification arises due to the attachment of a methyl group to the -5-position of cytosine by DNA (cytosine-5-)-methyltransferase without changing the original DNA sequence (Ball et al., 2009; Suzuki and Bird, 2008). DNA methylation not only plays an important role in gene regulation but also is crucial for maintaining genome stability. Most methylated CpG dinucleotides are located in repetitive DNA elements which make up to 45% of the genome. Methylation-free CpG dinucleotides are clustered in short stretches of DNA (500 bp to a few kb) known as CpG islands (CGIs) which are characterized by relatively high CpG density and located in the proximal promoter region of approximately 75% of human genes (Jones and Takai, 2001; Illingworth and Bird, 2009; Jones, 2012). Changes in DNA methylation patterns include global hypomethylation across the genome, gene specific hypermethylation/hypomethylation and loss of imprinting. The aberrant hypermethylation of cytosines in CGIs of active promoters is one of the main mechanisms of gene inactivation.

A variety of genotoxic agents are known to alter DNA methylation patterns (Hou et al., 2012; Merrifield and Kovalchuk, 2013). The induction of epigenetic modifications by radiation has been shown in experiments using cell cultures and different animal models (Antwih et al., 2013; Aypar et al., 2011; Goetz et al., 2011; Koturbash et al., 2006; Pogribny et al., 2005; Loree et al., 2006; Raiche et al., 2004; Kovalchuk et al., 2004; Lima et al., 2014; Wang et al., 2014; Bernal et al., 2013; Nandakumar et al., 2011). To date, there are several investigations in which promoter hypermethylation induced by in vivo radiation exposure of animals were evaluated (Kovalchuk et al., 2004; Lima et al., 2014; Wang et al., 2014; Bernal et al., 2013; Nandakumar et al., 2011). Radiation-

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induced methylation modifications have been suggested to potentially play roles in the mechanisms of genomic instability and transgenerational effects (Merrifield and Kovalchuk, 2013).

A number of important observations on epigenetic modifications in human tissues have been reported during recent years. Firstly, DNA methylation patterns (total hypomethylation and gene-specific hypermethylation/hypomethylation) either in white blood cells or other tissues have been found to be age-related. In genome-wide investigations, the preferential age-associated hypermethylation within CGIs of gene promoters was demonstrated (Bell et al., 2012; Johansson et al., 2013; Horvath, 2013; Day et al., 2013; Hannum et al., 2013; Yuan et al., 2015; Reid et al., 2012). Secondly, promoter hypermethylation of certain genes has been associated with the development of different disorders, including age-related diseases of neoplastic and non-neoplastic nature (cardiovascular diseases, neurodegeneration, metabolic and autoimmune disorders) (Van Otterdijk et al., 2013; Bell et al., 2012; Hannum et al., 2013; Yuan et al., 2015). Hypermethylation of certain loci was found to be largely shared by multiple tissues, not limited to the affected ones, and could be also observed in normal cells of the body such as blood leukocytes (Flanagan et al., 2009; Al-Moundhri et al., 2010; Tahara et al., 2013; Lakshmi Sana et al., 2013; Kim et al., 2013). Thus, assessment of radiation-induced aberrant hypermethylation that potentially could be a cause and/or a biomarker of premature aging of irradiated organism and/or of the development of age-associated diseases is an important task. However, little is known about CGI hypermethylation following radiation exposure of human body.

In this study we investigated hypermethylation status of promoter of genes with basic protective functions in the cells, the *RASSF1A, p16/INK4A, p14/ARF* cell cycle genes and *GSTP1* involved in xenobiotic detoxification, in blood leukocytes long term after radiation exposure in an attempt to discriminate age- and radiation-related changes.

## 2. Materials and methods

### 2.1. Subjects

In the present work, 208 unirradiated volunteers and 124 irradiated subjects (83 Chernobyl Nuclear Power Plant clean-up workers, 21 nuclear workers, 20 residents of territories with radioactive contamination, including 4 persons evacuated in 1986 from the Chernobyl zone) were enrolled (Table 1). Characteristics of the examined groups of clean-up workers (so-called "liquidators") and nuclear workers were described in detail previously (Kuzmina et al., 2014).

Briefly, the liquidators participated in ChNPP clean-up work within the 30-km zone in 1986–1987. The duration of work varied from 2 to 6 months, and individual doses ranged from 30 to 480 mSv (physical dosimetry). For about one-third of liquidators, radiation doses were not available. The duration of work of nuclear specialists with tritium and tritium oxide varied from 3 to 46 years; cumulative doses ranged from 11 to 994 mSv. The time between the end of work of with tritium and tritium oxide and sampling was more than 10 years for 48% of nuclear workers. Twenty examined individuals are permanent residents of territories contaminated with radionuclides (Novozybkov town and Novozybkov district, Bryansk region, 135–688 kBq/m$^2$ of $^{137}$Cs) as a result of the accident at the Chernobyl NPP. During the incident at the ChNPP, these subjects were irradiated at the age 8–17 years old. The age of all irradiated subjects at the time of sampling was 24–78 years. Two hundred eight healthy unirradiated subjects, matched by gender, age and smoking to the exposed groups, were enrolled as a control group (Table 1).

It should be noted that the exposed group was heterogeneous in terms of radiation qualities (such as e.g. dose and dose rate), and duration of and time after exposure. However, all these subjects had radiation history and therefore were combined in the exposed group in this study.

In addition, 74 offspring (2–51 years old at sampling) born to irradiated parents were examined. Among them, 29 were non-exposed children (born in 1987–2006) whose fathers were irradiated liquidators, 29 offspring born to irradiated fathers- nuclear workers, were enrolled. The time between irradiation of fathers and conception of children varied from few months to 18 years. Also enrolled were 16 children born after the Chernobyl accident in 1987–2005 from irradiated parents who had been exposed to maximal dose in 1986 and lived for a long time on contaminated territories (135–688 kBq/m$^2$, $^{137}$Cs, Novozybkov town and Novozybkovsky district, Bryansk region, Russia) before conception of children were examined. Postnatal irradiation of these children was minimal. Among these children, 4 individuals born to parents

**Table 1**
Characteristics[a] of examined subjects.

| | Examined subjects | | | | | | |
|---|---|---|---|---|---|---|---|
| | Irradiated subjects $n=124$ | | | Offspring born to irradiated parents $n=74$ | | | Unirradiated subjects[b] (control group) $n=208$ |
| | ChNPP clean-up workers $n=83$ | Nuclear workers $n=21$ | Permanent residents of territories with radioactive contamination $n=20$ | Born to clean-up workers $n=29$ | Born to nuclear workers $n=29$ | Permanent residents of territories with radioactive contamination $n=16$ | |
| Age, years | 38 to 76<br>54.81 ± 1.14<br>24 to 77<br>54.03 ± 1.15 | 53 to 77<br>67.29 ± 1.30 | 24 to 50<br>36.9 ± 1.53 | 3 to 19<br>13.21 ± 0.83<br>2 to 51<br>21.9 ± 1.64 | 17 to 51<br>37.69 ± 1.38 | 2 to 17<br>9.03 ± 1.27 | 19 to 77<br>37.03 ± 1.26 |
| Gender, male/female | 81/2<br>116/8 | 21/0 | 14/6 | 13/16<br>38/36 | 14/15 | 8/8 | 117/91 |
| The time between the end of radiation exposure and sampling, years | 17 to 21<br>19.05 ± 0.76 | 2 to 46<br>13.4 ± 3.17 | 0 | – | – | – | – |
| Accumulated doses, mSv | 30 to 480<br>183.8 ± 17.5 | 11 to 994<br>183.5 ± 52.7 | The individual doses are not known | – | – | – | – |

[a] The range of variability and mean ± SEM are demonstrated.
[b] The parents of these subjects had no history of radiation.