

# Blood cell gene expression profiles: A narrative review of biomarkers and effects of low-dose ionizing radiation exposure

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## Abstract

Ionizing radiation (IR) is a ubiquitous environmental agent whose effects on organisms are well known. This review provides a summary about definitions and man-made low-dose ionizing radiation (LDIR) sources and dosimeters used in radiation protection. Moreover, the main purpose of this article was to overview the pro-oncogenic effects of LDIR, and to provide experimental evidence that reinforce the use of gene expression data as biomarkers of LDIR effects. Our review showed that basic studies on biological response to LDIR are considered priority. Further, understanding occupational exposure to LDIR may provide valuable information to organize the prevention and prevent from the onset of long-term health effects in radiation workers. Currently, the biodosimetry-based assessment in certain high-risk occupational groups may be performed by using peripheral blood cells as samples for testing and validation of biomarkers specificity and sensitivity. Most of the studies on this topic are aimed at establishing new biomarkers and approaches to biological dosimetry, for allowing non-invasive monitoring of long-term health effects of LDIR. Analysis on changes in gene-expression, which is an early specific biological response to LDIR, could provide rapid estimates of individual dose in occupational cohorts, improving the management of periodical medical examination in subjects exposed to LDIR sources.

**KEY WORDS:** Ionizing radiation; low dose; occupational health; biological dosimetry; gene expression; PMBC.

## INTRODUCTION

Ionizing radiation (IR) is a ubiquitous environmental agent whose effects on organisms are well known [1]. The International Commission on Radiation Units and Measurements (ICRU) emphasizes the differences between the interaction of charged and uncharged radiation with matter dividing IR in two categories: directly IR and indirectly IR, which correspond to charged (i.e as  $\alpha$  and  $\beta$  particles) and uncharged particles (i.e as x-ray,  $\gamma$  particles and neutron), respectively [2].

All living organisms are continually exposed to various natural or man-made sources of IR. As well defined by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) [3], natural sources of IR mainly include the terrestrial radiation, and cosmic radiation. The first one comes from the earth, while the second one comes from the sun and outer space. Man-made sources of IR are mainly medical procedures. The World Nuclear Association [4] report-

ed that man-made IR currently accounts for approximately 20% of the worldwide radiation exposure, while the remaining 80% is ascribable to natural sources. During the past century, we observed a growing contribution of nuclear installations, nuclear weapon tests and applications of radionuclides in industry and medicine to man-made sources of IR exposure.

In addition to natural sources, medical exposure for both patients and workers is characterized by exposure to low dose IR (LDIR), which makes difficult to evaluate the related-cancer risk in the context of the risk assessment process. Furthermore, many individuals are exposed to LDIR for occupational reasons. For this reason, it is important to characterize a dose-response curve in order to explain and shape the health effects and to evaluate the risks of repeated exposure to low doses of IR.

In the (very) low dose regimen, responses to IR are more diverse and difficult to predict

### TAKE-HOME MESSAGE

*Early biomarkers identification is necessary to evaluate potential health effects and risk assessment of repeated exposure to low doses ionizing radiation.*

**Competing interests** - none declared.

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and individual susceptibility is prominent. Indeed, the relationship between absorbed dose, DNA damage, and health risk is an ongoing topic of debate.

Currently, there is a paucity of scientific evidence on the health effects due to LDIR exposure and the statistical power of epidemiological studies is quite insufficient to demonstrate the existence of an increased carcinogenic risk [5]. Moreover, the probabilities of experiencing detrimental effects from exposure to LDIR are estimated by mathematical extrapolation of data from those exposed to high-dose ionizing radiation (HDIR), on the basis of a linear no-threshold (LNT) model [6]. Studies based on the LNT model suggest that in the low dose range, radiation doses greater than zero increase the cancer risk and the risk of heritable diseases in a simple proportionate manner [7]. Conversely, the radiation protection standards established on the basis of current scientific evidence suggest that risks due to exposure to LDIR could be underestimated [4, 8, 9]. Indeed, the risk assessment depends on the knowledge of potential health effects of LDIR and on the mechanisms employed to predict them. The Radiation Protection International and National Agencies have established specific criteria and procedures for decreasing radiation exposure and minimizing unwanted effects in all different scenarios. According to this assumption and thanks to an increased awareness of molecular and cellular mechanisms of LDIR-induced biological effects, it has been possible to reach international consensus guidelines with the aim to minimize the risk of health effects using IR for medical, scientific and industrial purposes [10].

Prerequisite for planning any preventive intervention is the knowledge of the absorbed dose. In this context, physical and biological dosimetry are key tools for monitoring the individual absorbed dose of IR. However, the current physic and biological dosimetry approaches are not tailored to distinguish the early responses and long-term pro-oncogenic effects of LDIR, therefore, the discovery of inherent biomarkers represents a priority

area. Modern high-throughput technologies have sped up the discovery process and may give broader insight into biological events that follow the exposure to IR [11, 12]. Furthermore, gene expression profiles could be used as biological signature of dose received, providing new potential biologically-based dosimetry tools that could be used to optimize health protection guidelines governing the surveillance for occupational and medical use of LDIR [13].

The present narrative review introduces basic considerations and definitions about pro-oncogenic effects of IR, man-made LDIR, sources and type of dosimeters, followed by an overview of the studies on blood cell response to LDIR exposure performed by gene expression analysis.

## RESULTS AND DISCUSSION

### *Pro-oncogenic effects of IR*

Based on the emergent scientific evidence of the literature, the International Commission on Radiological Protection (ICRP) has classified the adverse effects of radiation into two categories, namely deterministic and stochastic effects [7]. Deterministic effects, defined as 'tissue reaction' by ICRP, are largely due to significant cell damage or death following exposure to high doses of IR, which were mainly observed after radiation accidents or in patients undergoing radiotherapy. Tissue reactions occur if the absorbed dose is greater than a threshold value, and the severity of these reactions increases as the radiation dose increases. On the contrary, stochastic effects are generally generated by long-term exposure to low-level doses of IR. According to the ICRP-103 Report, stochastic effects are the result of mutation in somatic cells, whereas heritable diseases are the result of mutation in germ cells [7]. These effects have a probability of occurring that is proportional to the absorbed dose yet without a threshold value, thus its severity is independent from the absorbed dose [14].

It is well-known that IR exposure is associated with the development of different type

of cancers including thyroid malignancies and leukaemia in children and adults [15]. It has been observed that IR can also increase the risk of cancer in nuclear industry workers exposed to very low IR rate [16]. However, experimental and epidemiological evidence demonstrates that a dose-response association with cancer or other diseases is not estimable, and that the LNT model may be misrepresentative of LDIR exposure [17]. A plausible explanation of the possible failure of LNT model is that it is based only on targeted effects of LDIR on the DNA molecule, which may directly result in DNA damage, cell death or other genetic aberrations [18]. In fact, there are also non-targeted effects of LDIR, such as adaptive responses, radiation-induced bystander effects and individual hypersensitivity to IR, which may strongly contribute to biological effects adding further doubts about the efficacy of LNT model [19, 20]. Adaptive response refers to the phenomenon by which cells irradiated with a low priming dose of IR may become refractory to a subsequent challenge with high dose of radiation [21]. The bystander effect refers to the behaviour of non-irradiated cells, neighbours of directly irradiated cells, which can exhibit effects of irradiation due to the exchange of information via intercellular communication [21]. Individual hypersensitivity to LDIR is a cellular phenomenon that mainly occurs in cells with a genetic predisposition (i.e., variants in genes involved in the response to radiation-induced DNA damage) [22, 23]. Moreover, the attention of scholars is increasingly drawn to the importance of non-genetic changes in cancer and non-cancer pathologies. These changes include those mediated by modifications of DNA (such as methylation), as well as modifications of histones at multiple positions (including phosphorylation, methylation and acetylation) and other DNA-binding proteins, and changes in expression of non-coding RNAs; all of which can regulate chromatin state and gene expression. DNA damage inflicted by chronic LDIR is very different. It is unclear whether there is a threshold of DNA damage that must be breached for

cells to respond, and whether continuous and consecutive DNA damage will be tolerated or ignored by the cell. It has been suggested that persistent DNA-damage signalling can drive cells into senescence. Nevertheless, the LNT model could not take into account several other factors such as age, gender, lifestyle, inflammatory responses, and oxidative stress [24]. As suggested by the European Low Dose Research towards Multidisciplinary Integration (DoReMi) project, the use of potential radiation biomarkers adequately validated in large IR epidemiology studies could be a step forward to assess LDIR-related pro-oncogenic effects [25, 26].

### *Sources of LDIR*

#### *General concepts*

Radiation exposure to humans can be categorised in external, and internal exposure; this type of exposure frequently occurs in medicine, when radioactive tracers are placed inside the body for diagnosis or therapy [27]. Medical diagnostic or therapeutic procedures account for about the 98% of all anthropogenic sources of radiation exposure, while only a small amount is due to other human activities involving radioactive material [28]. This data refers to worldwide average annual effective dose stated by UNESCEAR 2008, which estimates that people receive a total annual average dose from all sources of radiation of 3 mSv. The UNESCEAR also assumes that depending on different geographic areas and the level of health-care system, this value can vary within a range of 1-10 mSv [28].

#### *Natural sources*

Natural sources include cosmic and terrestrial IR. Cosmic and terrestrial radiation refer to the radiation from outer space and from primordial radionuclides contained within the earth's crust and core, respectively. Worldwide, naturally occurring radiation coming from the surrounding environment also encloses inhalation and ingestion of radioactive substances. Radon gas, generated by the decay of uranium 238, is widely distributed in the earth's crust and it may be found in outdoor,



indoor, soil air and drinking water. Moreover, food and drink may be naturally or, to a lesser extent, artificially, contaminated with radioactive isotopes including potassium-40, uranium-238 and thorium-232. UNSCEAR estimates that the annual global average effective dose from natural background radiation is about 2.4 mSv per person. This value may vary depending on geographic location and soil composition. The UNSCEAR calculates that every person worldwide annually receives an effective dose of 0.3 mSv from cosmic sources, 0.48 mSv from terrestrial sources, 1.3 mSv from radon and 0.3 mSv from ingestion of food and drink (Figure 1A) [28]. Annual effective dose from radon exposure is approximately half of the entire effective dose coming from the all other natural sources [29].

#### *Medical sources*

Over the years, diagnostic radiology, nuclear medicine and radiation therapy have evolved into more and more sophisticated techniques, which now allow a more effective diagnosis and therapy for different types of diseases. Routine dental X-rays are among the most common sources of IR exposure for healthy individuals globally, with 300 examinations/1000 individuals/year as documented by the UNSCEAR. UNSCEAR has estimated the average annual effective dose from medical applications of IR in industrialized countries at 1.9 mSv and in non-industrialized countries at 0.32 mSv. For instance, among the US population the annual per capita average effective dose from medical sources is about 3 mSv, resulting in radiation dose comparable to that from natural background. In Kenya, these values considerably decrease to only 0.05 mSv per person [28, 30, 31].

The largest source of IR used in medicine are the X-ray machines mainly employed for diagnostic radiology. The annual dose due to conventional X-ray diagnostic radiography has been approximately calculated closed to 0.3 mSv (Figure 1B) [28, 30, 32]. In addition to standard X-ray radiography, during the last two decades, several advanced tools, such as computed tomography (CT) and fluoroscopy,

have been developed to improve diagnostic/therapeutic application of IR. CT scanner account for about 1.5 mSv (Figure 1B), corresponding to 43% of the total collective dose due to X-ray exposure [28, 30, 32]. Interventional cardiologists have a high exposure rates to X-ray, which was estimated in the U.S. at the annual effective dose of approximately 0.4 mSv (Figure 1B) [28, 30, 32]. Nuclear medicine approaches are additional medical sources of IR that account for an annual dose close to 0.8 mSv for the US population (Figure 1B) [28, 30, 32].

The employment of the medical LDIR sources have a dual impact on human health involving both workers and general population. For this reason, the use of these IR sources in clinical practice is regulated by national and international recommendations and guidelines that take into account the benefit/risk ratio.

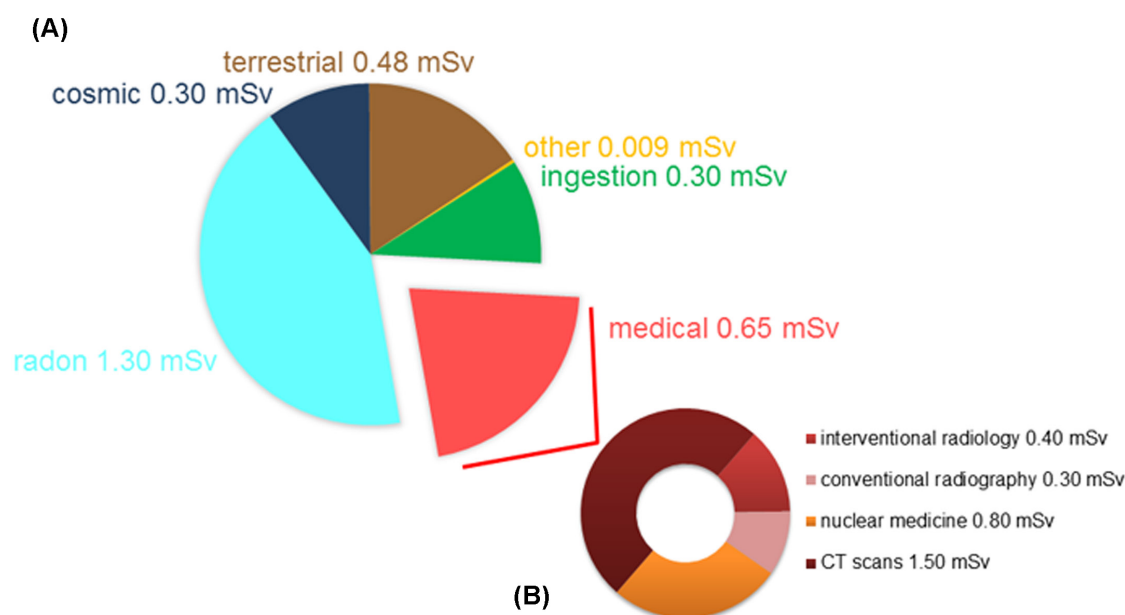
#### *Current dosimetry approaches and applications*

##### *General concepts*

In order to guarantee an adequate level of radiation protection and to ensure a safe and acceptable employment of IR sources, the measurement and calculation of radiation doses quantities (dosimetry) from these licensed activities is of fundamental importance. The dosimetry can be categorized in physical and biological dosimetry. In order to assess the dosimetric quantities, the first one uses instruments (dosimeters); whereas the biological dosimetry measures the biological changes induced by radiation [33].

##### *Physical dosimetry*

Generally, in medical exposure, particularly in hospital, the measurement of IR for radiation protection purposes is mainly engaged in two distinct experimental conditions. The first of them concerns the extent of the basic dosimetric quantities such as absorbed dose, kerma, and exposure, whereas the second concerns the assessment of activity (or concentration of activity) of radionuclides [34]. Both types of



**Figure 1.** Pie chart representation of the average annual effective dose from natural, artificial and medical radiation sources in US. A) All sources of LDIR and B) Specific medical source of LDIR expressed in mSv (Source: NCRP Report 160).

measurement have a common purpose, which is to provide the necessary test data to determine the protection quantities, such as the equivalent dose ( $H_T$ ) and the effective dose (E) which are associated, in accordance with the radiation protection legislation, with the limit values for exposure of population and workers to IR [7, 35].

In the hospital setting, workers are involved in two different branches of the dosimetry, namely the workplace monitoring and the individual monitoring. Individual monitoring is the measurement of radiation doses received by individual workers. The methods available generally require that the radiation sources and the potentially exposed workers have to be identified. Workplace monitoring is carried out where there is a potential exposure. Workplaces are designated as controlled areas if specific protection measures or safety provisions are, or could be, required for controlling normal exposures or preventing the spread of contamination during normal working conditions, and preventing or limiting the extent of potential exposures [7].

Different types of radiation-monitoring instruments have been extensively evaluated in the last years, as detector devices to control

and to measure worker and public exposure to IR. These special methods of estimating radiation doses are usually constituted by a radiation detection system and a signal processing system provided by the detector. Depending on the type of detector used, the dosimeters can be classified as active and passive [36]. The active dosimeters provide a direct display of the accumulated dose as well as some additional functions such as alarm threshold settings for dose or dose rate values. They are particularly useful for measuring real-time exposure to IR and they are employed for complementary dosimetry in the case of high radiation. The passive dosimeters do not provide direct readouts but they are used to estimate the effective dose received by the workers. Passive dosimeters are worn by users for a specific period during which information related to radiation-induced signal are recorded and stored. The data are thus processed and analysed later. The effective dose is calculated by dosimeters worn between the waist and the neck. These dosimeters are often called whole-body dosimeters. In addition, extremity dosimetry system worn on fingers or wrists or near the eye, are also used to measure equivalent dose to these tissues.

The advantages in using a personal dosimeter is that it provides more realistic estimation of radiation absorbed by operator [34].

The dosimeters employed in radiation protection (both for the monitoring of the area and individuals) use a wide range of detectors depending on the measurement for which they are provided. In relation to the type of radiation to be detected, one can distinguish three main categories of dosimeters: dosimeters for X and gamma radiation, for beta particles, and for neutron. The last one could be categorized in dosimeters for thermal and fast neutrons [37, 38].

### *Biological dosimetry*

As biological dosimetry we refer to indirect methods to estimate absorbed doses of IR by evaluating the biological effects occurring after exposure. Therefore, in the last years we witnessed the increase of research interest in the identification of potential biological biomarkers that mirror the adsorbed IR dose and that could be reliable biological dosimeters in large population studies [33, 39, 40]. They can be used as biological dosimeters to evaluate the response to IR, particularly in occupationally exposed individuals. There are some parameters that have to be considered in the choice of a potential biological dosimeter. A good biological dosimeter has to be easily obtainable and measured on tissues or fluids, being IR-sensitive, with changes linear to dose-response, and eventually it should be able to distinguish acute and/or chronic exposure.

The current biological dosimeters are mainly based on the assessment of chromosome damage or aberrations, gene activation and biophysical changes in tissues [41].

Among the most radiosensitive cells, there are lymphocytes, and their count after whole-body radiation exposures could be an easily available bio-indicator of damage. Under exposure to IR dose range between 2-3 Gy and 8 Gy, the number of circulating lymphocytes declines approximately by 50% over 12 h [33]. Moreover, it is currently accepted that DNA damage repair mechanisms are mainly

a mark of responses to high doses of IR, while cellular responses such as dysregulation of cytokine levels, and changes in the transcriptome and proteome are involved in response to LDIR [42].

Pernot et al [25, 26] proposed an interesting classification of LDIR biomarkers for epidemiological studies undertaken by the work of the Low European Dose Research towards Multidisciplinary Integration (DoReMi) network that is summarized in Table 1. According to this classification, the biomarkers that can potentially become useful biological dosimeters include: (i) cytogenetic biomarkers; (ii) biomarkers related to germline and somatic mutations; (iii) biomarkers related to nucleotide pool and DNA damage; (iv) biomarkers related to transcriptional and translational changes (Table 1).

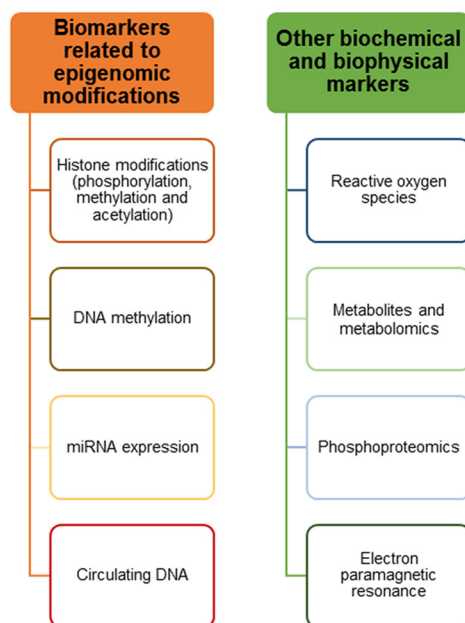
Compared to classical cytogenetic assays, which are time consuming, changes in gene expression of a small number of selected genes would result in an easy, quick and specific approach for monitoring LDIR exposure in humans [25, 26]. However, gene expression analysis has the disadvantage to be short-lived and changes in the expression are not specifically correlated yet to LDIR. Thus, in response to the need of novel biomarkers that are sensitive to incremental changes in dose, stable for days after exposure and replicable, the interest on the use of epigenomic and metabolic profiling as potential biological dosimeters has grown [25, 26]. In Figure 2 are reported the most innovative biomarkers applied for the evaluation of biological effects after LDIR in large epidemiological studies, as well as biomarkers related to epigenomic modifications and biochemical and biophysical markers.

As basic studies on the biological response to LDIR are considered a research priority in order to better understand the occupational risks associated with working in radiation departments with the possible development of long-term health effects, non-invasive or mini-invasive techniques that pose no health risk for the individuals are required. However, the radiation sensitivity of cells is different, and

**Table 1.** Biomarkers for monitoring biological effects of LDIR (Source: Low European Dose Research towards Multidisciplinary Integration (DoReMi) network, modified) [25].

Type of biomarkers	Assay	IR dose range	Time for detection of response (hours, days, years)
Blood cell count	Count of peripheral blood lymphocytes	from 2/3 to 8 Gy	12-24 hours
<i>Cytogenetic</i>			
• Dicentric chromosomes	• Dicentric chromosome	• from 0.1 to 5 Gy	Years
• Chromosome translocations	• fluorescence in situ hybridization (FISH), chromosome banding	• from 0.25 to 4 Gy	
• Premature chromosome condensation		• from 0.2 to 20 Gy	
• Complex chromosomal rearrangement		• NA	
• Telomere length		• NA	Months
• Micronuclei	• Flow cytometry, FISH, qPCR	• from 0.2 to 4 Gy	
<i>Gene mutation related</i>			
• Single nucleotide polymorphisms (SNP)	• SNP assay/genome wide association studies (GWAS)	NA	
• Copy number variants and alterations	• Comparative genomic hybridization (CGH), FISH, next generation sequencing (NGS)	NA	Years
• Induced somatic mutations:	• Flow cytometer assay for Glycophorin A	>1 Gy	
	• PCR for hypoxanthine-guanine phosphoribosyl transferase mutation	>90 mGy	
<i>Related to nucleotide pool and DNA damage</i>			
• Double and/or single strand break	• Comet assay	from 0.1 to 8 Gy	Weeks
• $\gamma$ H2AX assay	• Immunofluorescent staining, flow cytometry, high throughput techniques	from 0.01 to 8 Gy	Days
• Extracellular 8-Oxo-deoxyguanosine	• HPLC-enzyme-linked immunosorbent assay (ELISA), ELISA	from 1 to 100 mGy	Weeks
<i>Related to transcriptional and translational changes</i>			
• Gene expression genes (cell cycle, apoptosis and DNA repair)	• TaqMan assay, qPCR, microarray, nanostring, NGS	NA	Months
• Serum amylase	• Serum amylase test	>1 Gy	Days
• C-reactive protein	• ELISA	>1 Gy	Years
• Cytokine levels	• ELISA	>m1 Gy	
• Protein analysis	• Western blotting, ELISA, high throughput techniques	NA	





**Figure 2.** Novel discovered potential biomarkers for epidemiological studies on LDIR effects.

today, the biological dosimetry assessment for individual LDIR exposure in risk categories, such as occupational workers and patients underwent to radiation therapy treatment, is mainly performed by using peripheral blood cells as samples for training and validation of biomarkers specificity and sensitivity. Among many attempts to improve biological dosimetry tools, studies on changes in gene-expression profiles in peripheral blood cells could provide rapid estimates of individual dose in population studies improving the management of periodical medical examination in workers exposed to LDIR sources.

### *Gene expression profiles in human blood cells as biological dosimetry for LDIR*

#### *In vitro studies in human blood cells*

One of the first studies aimed at identifying LDIR-responsive genes was conducted by comparing the results of whole blood gene response to low dose with those at high doses of IR. Amundson et al [43], using a human myeloid tumour cell line (ML-1) exposed at total doses of 0.02-0.50 Gy, found that low dose-rate irradiation produced, after 2 hours, two different gene expression patterns analysed by

microarray analysis. In particular, the authors reported a dose rate-dependent induction of a group of genes involved in apoptosis control, such as GADD45A and CDKN1A; and a dose rate-independent of a group of genes mainly involved in cell cycle regulation, such as MDM2 [43].

A cDNA microarray technology was used to evaluate the global transcriptional changes in human lymphoblastoid AHH-1 cells after 4 hours from the exposure to 0.05, 0.2, 0.5, 2.0 and 10-Gy doses of  $\gamma$ -rays [44]. The authors observed an equal distribution of up- and down-regulated genes in the 0.05-, 0.5- and 2-Gy sets, but the early alteration of the expression of specific genes was dose-dependent. The irradiation at 0.05 Gy induced up-regulation of 25 genes, some of which are involved in signal transduction (BMPR2, GPR124, JIP2/MAPK8IP2 and AGGF1), intercellular transport/cell-cell communication (ANXA13, Connexin 43), and ion channeling/neurotransmission (GRIA3/GLUR3). At 0.2-Gy the expression changes occurred in genes involved in signal transduction pathways (RASAL2, KLRC2, VTN and PLXNA4), cytoskeleton and cell movement (PRICKLE2, ADAMTS1 and

RSHL3), cell adhesion (LTBP2, (PCDHA9 and PCDH18), and DNA-binding factors (RNF2 and UBR4). Doses from 0.05-10 Gy showed a clear dose-dependent induction of the DNA repair gene XPC and tumour protein p53 inducible protein 3 gene (TP53I3). This study provides evidence of some radiation-response genes that can be recognized as potential biomarkers of radiation exposure [44].

A first study on human primary cells was performed on lymphocytes isolated from four healthy volunteers exposed to *in vitro* irradiation with  $\gamma$  rays dose of 0.1, 0.25 or 0.5 Gy. Microarray analysis performed on samples collected 48 hours post-irradiation, revealed dose-dependent expression changes in different type and number of genes. The LDIR (0.1 Gy) caused the modulation of 86 genes, while the HDIR (0.25 or 0.5 Gy) induced expression changes in 130 and 142 genes respectively. Among all the three doses of irradiation, the authors identified a set of 34 common genes that were significantly modulated in lymphocytes, including the up-regulation of SERPINB2 and C14orf104 and the down-regulation of CREB3L2, DDX49, STK25 and XAB2. Moreover, the CYP4X1, MAPK10 and ATF6 genes, were exclusively modulated by 0.1 Gy, the DUSP16 and RAD51L1 genes by 0.25 Gy, and the RAD50, REV3L and DCLRE1A genes by 0.5 Gy, respectively. Finally, the authors showed that the exposure of human lymphocytes to LDIR rather than HDIR significantly affects biological processes/pathways as DNA repair and stress response, cell growth and cell differentiation, metabolism and transcription regulation [45]. Further advance in the understanding of LDIR effect in terms of gene modulation came from a work carried out by Gruel et al [46]. In this study, the authors hypothesized that different subsets of blood cells did not show the same gene expression response to radiation exposure. Furthermore, CD56<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> cells were isolated from peripheral blood of five healthy donors and exposed to 0, 0.05 Gy and 0.5 Gy of IR. After 24 hours from exposure to 0.5 Gy, the mi-

croarrays analysis showed the induction of BAX, PCNA, GADD45, DDB2 and CDKN1A in all cell populations. However, after 3 hours from the exposure to LDIR (0.05 Gy) the number of negatively modulated genes in CD4<sup>+</sup> T cells was 10 time greater than in CD8<sup>+</sup> and CD56<sup>+</sup> T cells, thus suggesting CD4<sup>+</sup> subpopulation as the most sensitive cells to LDIR. The analysis of the down-modulated genes in CD4<sup>+</sup> cells showed that the early biological response to LDIR in this subset of cells affected cellular processes mainly involved in protein biosynthesis and oxidative phosphorylation [46].

Paul and Amundson [47], found a 74-gene signature able to discriminate between four specific IR doses (0.5, 2, 5 and 8 Gy) after 6 and 24 hours from exposure of quiescent human peripheral blood lymphocytes. Interestingly, the highlighted genes mostly included those associated with p53 response. These data suggested gene expression as a useful biomarker for estimation of radiation dose exposure at both high and low dose rates [47]. Knops et al [48] provided a further study on gene expression profiling approach in human peripheral blood lymphocytes isolated by six healthy donors and irradiated with low (0.02 or 0.1 Gy) and high (0.5 or 4 Gy) doses of  $\gamma$  ray. Since the gene expression analysis exhibited well-defined physiological responses especially at low dose, the microarray expression profiling was suggested as accurate, sensitive, rapid and reliable biological dosimetry to predict acute low-dose exposure [48].

Two works by El-Saghire et al [49, 50], demonstrated that LDIR may cause modulation of both innate and adaptive immunity responses in whole blood samples. In the first study [49], the authors demonstrated by the Gene Set Enrichment Analysis (GSEA) that peripheral blood samples exhibited the activation of both a 'classical' radiation pathway characterized by the induction of gene involved in DNA damage and apoptosis in response to high doses of X-rays (1 Gy), and inflammatory pattern characterized by the induction of immune-related genes in response to low doses of X-rays (0.05 Gy). In a second

study, the same authors [50], found similar results in isolated human primary monocytes in response to LDIR (0.05 and 0.1 Gy) and HDIR (1 Gy). In particular, the validation by Real Time PCR assay showed that genes such as cell cycle arrest inducer gene (CDKN1A), pro-apoptotic gene (AEN), and DNA-damage and repair genes (POLH and DDB2) were up-regulated only in response to HDIR. Otherwise, the LDIR exposure caused up-regulation of genes associated with innate immunity including HMGB1, TLR4, TLR9, MyD88 and IRAK1. Taken together the two studies highlighted that LDIR and HDIR may induce different cellular responses in human peripheral blood cells, and that low-dose exposure mainly triggers immune-stimulatory and pro-survival responses [49, 50].

The relevance of changes in gene expression profiles as biomarkers of exposure to LDIR has been emphasized also by Manning et al [51]. In this study CCNG1, FDXR, and DDB2 genes have been recognized as key signatures of human blood cells after LDIR exposure (0.1 Gy). The transcriptional modifications induced by LDIR ranging from 0.005 to 0.1 Gy, displayed also a linear correlation with the dose [51].

More recently, a study by Ghandhi et al [52] attempted to shed light on the biological response to prolonged LDIR exposure. Human whole blood was irradiated with three LDIR doses (0.56, 2.23, and 4.45 Gy) using both acute dose rates, and low dose-rate. Over a 24-hour of continuous exposure, they were also to distinguish low dose-rate exposed samples from acute dose exposed samples, using a gene expression-based classifier, showing the possibility to identify a gene-based signature related to low dose-rate exposures for large-scale biological dosimetry. In particular, the authors found that RBM3 and GRM2 genes were up-regulated and DUSP3 and ID2 were down-regulated by LDIR only and not acute [52].

Other literature studies [53] have aimed to identify specific metabolomic and lipidomic responses to radiation using animal mod-

els to determine which metabolites or lipids most frequently experienced perturbations post-ionizing irradiation (IR) in preclinical studies using animal models of acute radiation sickness (ARS) and delayed effects of acute radiation exposure (DEARE). These studies showed that clear changes in IR-induced injury were found in citrulline, citric acid, creatine, taurine, carnitine, xanthine, creatinine, hypoxanthine, uric acid, and threonine. Some of these metabolites may be ubiquitous and appropriate for use in diagnostic or prognostic biomarker panels.

A recent meta-analysis of high-throughput *ex vivo* analysis of gene expression, which was carried out on human peripheral blood exposed to low linear energy transfer (LET), led to the selection and subsequent validation of 6 genes involved in DNA damage and mitotic cell cycle checkpoint as DRAM1, NUDT15, PCNA, PLK2 and TIGAR. After 24 hours of *ex vivo* X-rays-irradiation, the mRNA levels were significantly increased at 1–4 Gy respect to non-irradiated controls. Interestingly, the PCNA expression showed dose-dependent upregulation [54].

The general results from *ex vivo* human blood IR exposure highlighted that it is possible to identify both dose-dependent and dose-rate-dependent different gene expression profiles. However, the general experimental setting provided analysis at very early time points after LDIR exposure, making it difficult to understand the true value of the data obtained and to assess a specific low dose risk.

### *Ex vivo studies in human blood cells*

Although several studies investigated how gene expression changes correlate with *in vitro* exposure of human blood cells to LDIR, the studies on gene expression profiles performed on *ex vivo* blood cells of occupationally or unintentionally exposed subjects are still poor. Albanese et al [55] analyzed the transcriptional changes of cytokine and their receptor levels in mononucleated cell isolated from peripheral blood of 19 healthy adults who lived near the Chernobyl Nuclear Power

Plant and were chronically exposed to LDIR ranging from 0.18 to 49 mSv over a period of 11 to 13 years. The authors reported a set of common genes expressed across all RI doses (genes encoding for serine/threonine protein kinase receptor, transforming growth factor receptor, EB13 and CD40 ligand) and distinct gene expression patterns (genes encoding for growth factors, cytokine receptors, and their cognate ligands, as well as for apoptosis-modulating proteins) in individuals exposed to LDIR greater than 10 mSv or less than 10 mSv [55].

Physicians, nurses and radiological technicians employed at hemo-dynamics, nuclear medicine and radiodiagnosis sectors exposed over a period of  $9.32 \pm 5.97$  years to radiation doses between 0.696 and 39.088 mSv and non-exposed workers were object of a gene expression profiling study by Fachin et al [56]. The cDNA microarray analysis detected a set of 78 differentially expressed genes in peripheral blood lymphocytes of 14 healthy exposed workers compared to 9 non-exposed subjects. Mostly of the *modulated genes* are implicated in DNA repair (RAD52, LIG3, ERCC5, XPA), stress response (DUSP22, GSTP1, PPP2R5A), and cell cycle proliferation/regulation (TGFB2, IL16, RHOA). Among these transcriptional patterns emerged that 21 genes were up-regulated (XPA and LIG3 significantly), while 57 were down-regulated (ERCC5, DUSP22, SEPT6 and RHOA significantly). This evidence highlights that chronic low level exposure to IR can induce stable transcriptional changes in the radiation workers [56].

In a similar study, differences in gene expression profiles between 28 healthcare professionals (physician and nurse) exposed to a persistent cumulative dose of very LDIR ( $19 \pm 38$  mSv) and 28 non-exposed subject were examined by Morandi et al [57]. The oligo-microarray approach revealed a set of 256 differentially expressed genes in peripheral blood mononuclear cells between the two groups. The genes were mainly associated to biological processes such as DNA packaging, chromatin scaffolding, nucleosome assembly,

mitochondrial electron transport NADH to ubiquinone (significant down-regulation of MTND). Additional analysis of gene expression data performed in a subgroup of 22 exposed individuals with  $> 2.5$  mSv effective dose revealed 156 differentially expressed genes compared to non-exposed subjects. The genes modulated in the subjects exposed to IR doses  $> 2.5$  mSv per year include the genes belonging to the same pathways found in the whole group of exposed and genes involved in programmed cell death (up-regulation of AHR, BIRC3, CLU, DUSP6, FADD, LGALS2, MAP3K5, MMD, NGFRAP1, SIRT1, TNFRSF10C), and in cation and iron homeostasis (MT1B, MT1E, MT1G, MT1X) [57].

All these findings highlight the relevance of expression profiles analysis as a potential powerful tool for detection and validation of dose- and time-dependent panels of 'radiation response' genes in radiation workers. Moreover, in this type of irradiated subjects, a reliable panel of biomarkers for evaluating the risk to develop IR-dependent cancer would be a crucial step forward. However, this issue has been point out only by a few number of studies. In fact, the validity of this approach as suitable, highly sensitive and rapid molecular screening test for IR biological effects is mainly provided by studies performed on patients IR exposed for medical purposes [58, 59]. A first relevant study, conducted on peripheral blood mononuclear cells of healthy donors and patients prior and after irradiation following total body irradiation with 150 cGy or 200 cGy, allowed to identify a set of 25 differentially expressed genes that may distinguish with an accuracy of 90%, healthy donors, non-irradiated patients, and irradiated patients [58]. In a second study, the same authors reinforced this data demonstrating that gene expression signatures in peripheral blood mononuclear cells can be specific, accurate over time, and not confounded by inter-individual difference [59].

A recent review [60] demonstrated increased residual DNA damage in radiosensitive individuals compared to normosensitive individ-



uals based on alteration at DNA repair foci or other sites coding for growth factors or cytokines that were connected to radiosensitivity in normal tissue.

As the medical staff represent the largest group of workers occupationally exposed to IR, a recent review [61] aimed to identify the genotoxicity biomarkers that are most elevated in IR-exposed *vs.* unexposed health care workers. It showed that chromosome aberrations and micronuclei frequencies were significantly different between IR-exposed and unexposed workers unlike other biomarkers of genotoxicity, confirming their relevance as genotoxicity biomarkers that are consistently elevated in IR-exposed *vs.* unexposed workers.

In the last years, our knowledge on transcriptional signature of radiation exposure comes from studies on patient underwent to medical procedures or treatments. Indeed, a gene expression study on peripheral blood cells IR exposed *ex vivo* and *in vivo* from patients with prostate cancer treated by radiotherapy described *FDXR* as the best biomarker of IR exposure and suitable for biological dosimetry in human blood [62, 63]. The study reported the expression changes of the ferredoxin Reductase gene (*FDXR*) as a sensitive and reliable tool for assessing radiation dose even after low doses of radiation. Moreover, the calculation of *in vivo* dose-response for estimated dose in blood samples from cancer radiotherapy patients and diagnostic CT showed that the endogenous expression of *FDXR* at 24 hours post-irradiation had a significant linear relationship with the physical dose estimate, suggesting *FDXR* gene modulations suitable for biological dosimetry in human blood.

Interestingly, a specific transcriptional signature associated with inflammatory processes was identified in a study on blood samples from 20 patients affected by different types of cancer underwent to Intensity Modulated Radiotherapy (IMRT). The gene set analysis (GSA) obtained 24 hours after the first fraction, just before the fifth or sixth fraction, and the last fraction highlighted the up-regulation of interleukin 18 family and Class I

MHC mediated antigen processing and presentation genes before the last fraction, at the last time. The relevance of such evidence lies in the possibility to identify a panel of ideal IR responsive genes, particularly those regulating the inflammation [64].

Finally, worthy of note is the result obtained from a study on the cellular and molecular long-term effects on patient undergoing neuro-interventional radiology procedures. The analysis of  $\gamma$ -H2AX foci and gene expression in blood lymphocytes revealed an evident DNA damage and altered gene expression. Among the analyzed genes, *CDKN1A*, *FDXR*, *BCL2*, *MDM2*, and *SESN1* also showed a linear relationship with IR dose [65].

## CONCLUSIONS

### *Future direction and implications for research and human health*

The biological effects and the cancer risk associated with the exposure to IR has been known for many years and derives mainly from groups of people who have experienced high doses of radiation exposure [15, 66, 67]. Our knowledge about the risks in cancer mortality/incidence following HDIR exposure has been well established after follow-up studies based on Japanese Atomic Bomb survivors [68]. The accident in the Chernobyl Nuclear Power Plant (CNPP) in 1986, and the nuclear power plant accident in 2011 at Fukushima widespread concerns about the acute but late effects of LDIR, such as cancer induction [69, 70]. However, there is little evidence of elevated cancer risks in groups exposed to medical source of IR, so that it may be premature to use these data to estimate LDIR risks for a general population.

The statistical power necessary to detect an adverse LDIR-dependent health effect especially in occupational settings requires a large number of exposed workers and a sufficiently long follow-up to account for the latency periods. Moreover, another limitation of many of these studies is a bias related to poor response rates on surveys that usually lacked age-matched controls and the exclusion of

non-physician allied health staff [71].

Different studies have showed that one of the major biological response to LDIR is the transcriptional response to DNA damage. Gene expression changes were established as an early indicator of cellular responses to LDIR [13]. The response of some genes to radiation appears to be dose-rate dependent while for other genes it appears to be dose-rate independent [43]. Thus, over the past few years, gene expression profiles, obtained using microarray technology, have also been used for biological dosimetry purposes. Most of these studies used blood cells for radiation doses between 0.5 and 10 Gy, over a period between 4 and 24 h. However, currently, no universal pattern of response has been identified and not all changes in gene expression levels correlate with radiation exposure time or dose.

Liquid biopsy that evaluates gene expression profiles in circulating tumor cells and/or peripheral blood mononuclear cells has recently emerged as a powerful technique for mini-invasive cancer diagnosis and some evidences highlight that circulating tumor cells could resemble those resident in a solid tumor [72]. In a recent study, Bazyka et al. demonstrated that the radiation exposure of Chernobyl shelter construction workers may influence the expression of cellular and molecular markers on lymphocytes [73]. All these findings highlighted that further studies of gene expression profiles changes in peripheral blood mononuclear cells of occupationally exposed workers could provide crucial information to estimate the real unhealthy dose and time of exposure to LDIR.

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