Radiazioni - Ricerca e Applicazioni

Vol XX n. 3 e vol. XXI n. 1, Dicembre 2017-Aprile 2018

Pubblicazione periodica quadrimestrale
Editrice: *Società Italiana per le Ricerche sulle Radiazioni*
Registrazione del Tribunale di Roma n. 406 del 6 Agosto 1998

Direttore Responsabile
Francesca Ballarini, Università degli Studi di Pavia e INFN-Sezione di Pavia
e-mail: francesca.ballarini@unipv.it Tel.: 0382 987949

SOMMARIO

Caratterizzazione del campo neutronico prodotto da ciclotroni medicali

D. Alloni*[1,2], M. Prata[1,2], B. Smilgys[1]

[1]LENA – Laboratorio Energia Nucleare Applicata, Università degli Studi di Pavia
Via Aselli 41, I 27100 Pavia, Italia

[2]INFN – Istituto Nazionale di Fisica Nucleare, Sezione di Pavia
Via Bassi 6, I 27100 Pavia, Italia

e-mail: daniele.alloni@unipv.it

Introduzione

Negli ultimi quindici anni, l'esame PET (Positron Emission Tomography) è diventato una diffusa tecnica di imaging funzionale per determinare i processi biochimici e fisiologici in vivo utilizzando radiofarmaci marcati con radionuclidi beta-emettitori, come ad esempio il ^{11}C, ^{13}N, ^{15}O e il più diffuso ^{18}F, e andando a misurare la radiazione di annichilazione mediante tecniche di coincidenza. I radionuclidi amministrati ai pazienti sono principalmente prodotti mediante ciclotroni commerciali.

Presso il Laboratorio Energia Nucleare Applicata (L.E.N.A.) dell'Università degli Studi di Pavia è in funzione un ciclotrone per la produzione di radioisotopi per uso medicale. Quotidianamente, da marzo 2016, nell'ambito di un accordo a carattere scientifico tra l'Università di Pavia e l'IRCCS Istituto Nazionale dei Tumori (INT) di Milano, il centro produce ^{18}F che viene poi sintetizzato all'INT per la produzione del radiofarmaco ^{18}F-FDG al fine di soddisfare la richiesta quotidiana di esami diagnostici PET. Da marzo 2016 a dicembre 2017 sono stati commercializzati circa 23 TBq di ^{18}F, corrispondenti a 816 forniture.

Il ^{18}F, con cui viene marcato il radiofarmaco, si ottiene mediante la reazione nucleare $^{18}O(p,n)^{18}F$, quando l'acqua arricchita in ^{18}O (arricchimento > 98%) viene bombardata con un fascio di protoni di energia ottimale di 18 MeV. Come prodotto di questa reazione si genera un campo secondario di neutroni ed un campo gamma dovuto alla diseccitazione dei radionuclidi formatesi durante la produzione di ^{18}F così come nelle reazioni indotte da neutroni nei diversi materiali che compongono il ciclotrone. Il campo neutronico così generato risulta essere la principale problematica da gestire in termini radioprotezionistici ai fini di garantire una adeguata schermatura in fase di installazione dell'acceleratore e per le problematiche legate ad un futuro programma di decommissioning. La conoscenza delle caratteristiche (intensità e spettro) del campo neutronico generato, insieme ai dati tecnici di funzionamento (ore di irraggiamento e intensità della corrente di fascio, materiali sostituiti etc.) e la classificazione dei materiali di cui è costituito il ciclotrone, permettono, infatti, di fare stime sull'attività indotta nei diversi materiali sia presenti nella macchina acceleratrice che nei materiali presenti nel bunker in cui essa alloggiata.

Di seguito verranno presentati i risultati di un confronto tra simulazioni Monte Carlo e misure sperimentali al fine di caratterizzare questo campo neutronico. I risultati qui riportati sono in parte nella referenza [1]. Il codice utilizzato per le simulazioni Monte Carlo è il codice MCNP6 [2], mentre la tecnica sperimentale utilizzata è l'analisi per attivazione neutronica (NAA) [3].

Materiali e metodi

Caratteristiche principali del ciclotrone e modellizzazione

Il ciclotrone installato al laboratorio LENA di Pavia è il modello IBA CYCLONE® 18/9, che accelera protoni con energia finale di 18 MeV e deutoni di 9 MeV.

La macchina è attualmente equipaggiata con tre bersagli, due dei quali per la produzione di ^{18}F (Large Volume e Small Volume) ed uno per la produzione di ^{13}N nella forma chimica di ammoniaca, con la possibilità di estendere ad otto il numero totale di bersagli. Il centro di produzione comprende un laboratorio di radiochimica dotato di una cella di manipolazione per il confezionamento della quantità richiesta di radioisotopo.

Per simulare il trasporto dei neutroni nel bunker e per modellizzare l'intera struttura del ciclotrone e dei locali è stato utilizzato il codice MCNP6.

Il target per la produzione di ^{18}F, rappresentato nel modello geometrico in Figura 1, alloggia il volume di acqua arricchita in ^{18}O ed è sottoposto a fascio di protoni con corrente nominale di 30 µA.

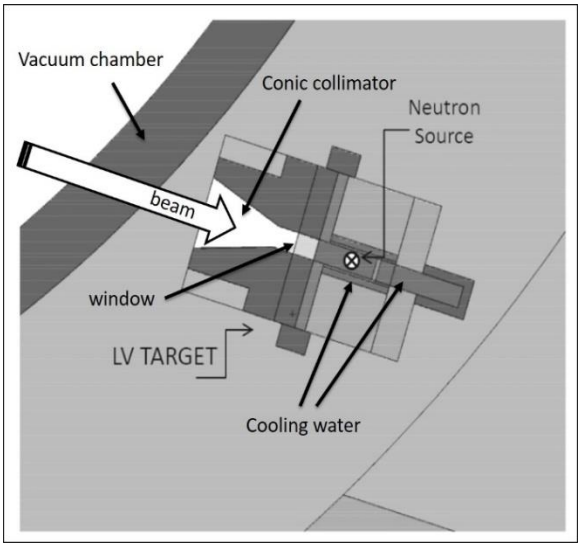

Figura 1: Dettaglio del target utilizzato per la produzione di ^{18}F.

In Figura 2 è visibile il modello geometrico costruito con MCNP6 dei locali, ed in particolare è visibile il bunker in cemento ordinario di densità 2.3 g/cm^3 che ospita il ciclotrone. Lo spessore medio dei muri è circa 200 cm. Una porta in cemento da tredici tonnellate anch'essa dello spessore di 200 cm consente l'accesso al bunker agli operatori nelle fasi di manutenzione ordinaria e straordinaria.

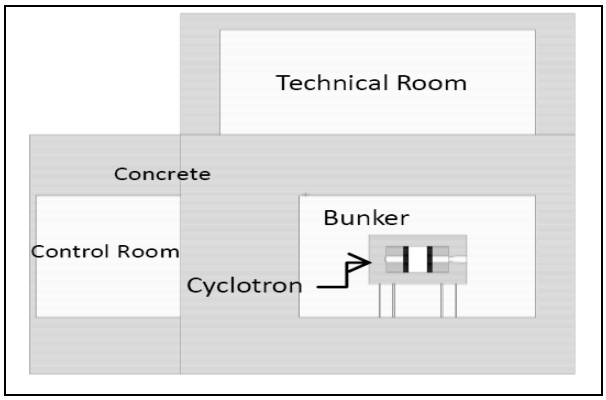

Figura 2: Modello geometrico dei locali in cui è visibile il bunker che alloggia il ciclotrone.

Le Figure 3 e 4 mostrano una vista dall'alto e laterale del modello geometrico. I punti in cui è stato valutato il flusso di neutroni e in cui sono state effettuate le misure sperimentali sono indicati con un cerchio crociato (LV, WALL e TOP).

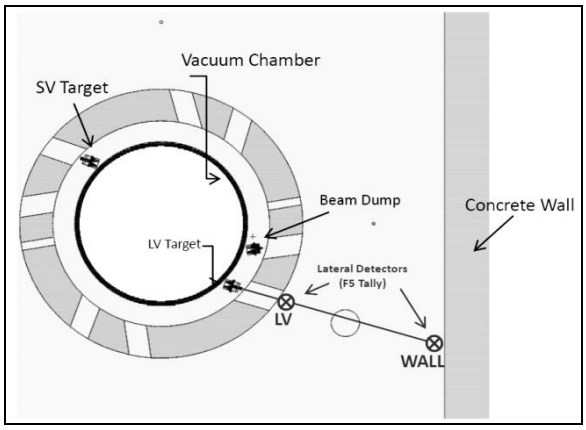

Figura 3: Vista dall'alto dell'acceleratore. I cerchi crociati rappresentano i punti in cui è stato valutato il flusso neutronico.

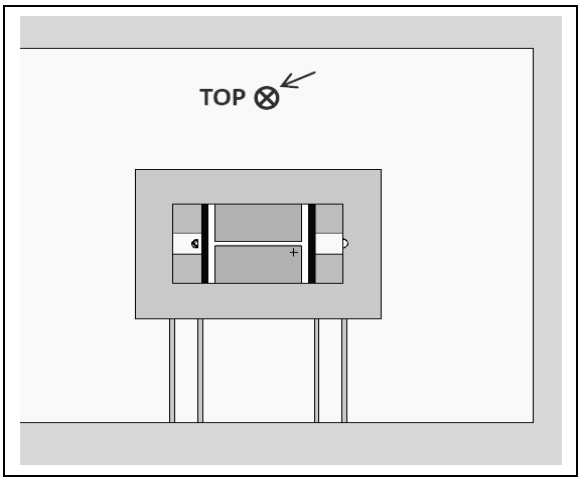

Figura 4: Vista laterale del modello geometrico del ciclotrone.

Il modello costruito ed utilizzato nelle simulazioni comprende in dettaglio sia la geometria sia le composizioni dei materiali che costituiscono il target, in modo da avere una precisa valutazione del trasporto all'interno dell'acqua arricchita e nella struttura del target stesso (vedi Figura 1).

Nelle medesime posizioni in cui è stato valutato il flusso con MCNP6 sono state successivamente effettuate le misure sperimentali con la tecnica NAA utilizzando targhette sottili.

Simulazione del trasporto dei neutroni

Lo spettro di neutroni intorno al ciclotrone è stato stimato simulando il trasporto dei neutroni partendo dal termine sorgente iniziale, che si origina dalla reazione nucleare $^{18}O(p,n)^{18}F$, attraverso il ciclotrone e i materiali che costituiscono il bunker. Il modello teorico più comunemente utilizzato per descrivere lo spettro neutronico sorgente è il modello ad evaporazione neutronica [4,5,6,7]. La formazione di nuclei composti ad energie di fascio inferiori a 20 MeV è il processo dominante di interazione nel quale il nucleo è in uno stato eccitato. Nel processo immediatamente seguente di diseccitazione, attraverso diversi canali di decadimento, il nucleo composto tende a raggiungere lo stato ad energia minima attraverso l'emissione di particelle (neutroni) descritto come un processo di evaporazione. La distribuzione di energia dei neutroni emessi può essere stimata dall'espressione seguente

$$N(E)dE \approx E^{0.45} \exp(-E/\alpha)$$

dove α è la temperatura nucleare, normalmente tra 1 e 10 MeV. Questa espressione è stata selezionata come input per lo spettro energetico della sorgente di neutroni nei calcoli di MCNP6 con α = 2.7 MeV [8].

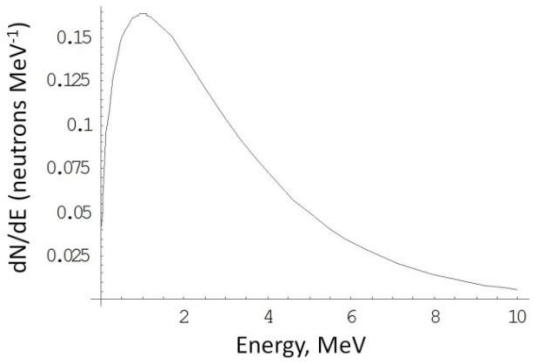

Figura 5: Spettro neutronico sorgente.

In questo modo essa rappresenta una sorgente isotropica puntuale di neutroni posizionata all'interno del target dove è localizzato il volume di acqua arricchita. L'assunzione di emissione isotropica di neutroni è un'assunzione sufficientemente accurata ai fini di calcoli radioprotezionistici, in particolare per calcoli di dose ambientale. Deviazioni dall'isotropia di emissione danno luogo ad una modifica dell'intensità e forma dello spettro soprattutto nelle immediate vicinanze del target ed in particolare di fronte ad esso.

Il numero di neutroni sorgente per ogni run di simulazione è stato di $5.0 \cdot 10^8$ in modo tale da ottenere un'incertezza statistica dal Monte Carlo inferiore all'1% in ogni posizione in cui è stato stimato il flusso.

Il flusso è stato calcolato in tre differenti posizioni: a 25 cm di fronte al target (LV), a 100 cm di fronte al target (WALL – vicino al muro del bunker) e a 180 cm al di sopra del ciclotrone (TOP). Il risultato del Monte Carlo è stato moltiplicato per l'intensità della sorgente di neutroni $I = 3.345 \cdot 10^{11}$

s^{-1} per ottenere lo spettro differenziale neutronico in ogni posizione (tale fattore tiene conto dell'intensità di corrente di fascio pari 30 μA).

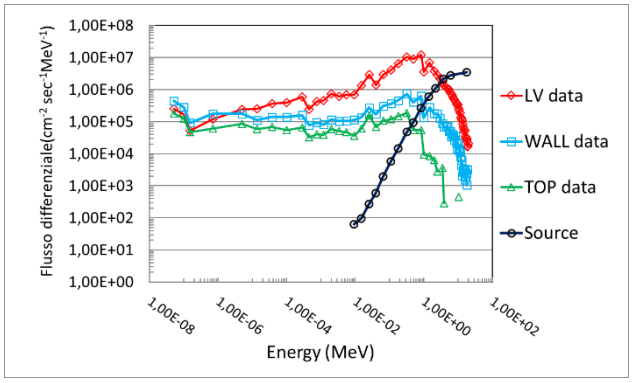

Figura 6: Spettri differenziali del flusso di neutroni nelle diverse posizioni (LV, WALL e TOP) e il termine sorgente ottenuti con il codice MCNP6.

Questi spettri, rappresentati in Figura 6, sono stati successivamente utilizzati, come spiegato nella sezione successiva, come spettri iniziali per il processamento dei dati sperimentali attraverso il codice di deconvoluzione SAND II **[**9,10,11].

Irraggiamento di targhette sottili e calcolo dell'attività indotta

Per validare i calcoli Monte Carlo sono state effettuate delle misure di flusso mediante la tecnica NAA e i dati sperimentali sono stati processati con il codice di deconvoluzione SAND II.

In breve, la NAA è comunemente utilizzata per determinare la concentrazione in tracce di elementi in varie matrici. Un campione viene irraggiato da un campo neutronico e vengono ad originarsi dei nuclidi radioattivi attraverso differenti reazioni nucleari. Questi decadono a loro volta emettendo radiazione gamma la cui energia è una caratteristica di ciascun radionuclide. Un confronto tra l'intensità della radiazione gamma emessa dal campione e quella di uno standard irraggiato nelle medesime condizioni permette una misura quantitativa della concentrazione dei vari nuclidi. Al contrario, se la composizione dei campioni è nota, una volta determinata la l'attività indotta è possibile risalire all'intensità di flusso neutronico e con sufficiente precisione al suo spettro.

Per le misure del campo neutronico del ciclotrone sono state scelte tre posizioni rappresentative. Attraverso reazioni nucleari di seguito riportate per la determinazione del flusso,

$^{187}Au(n,\gamma)^{198}Au$

$^{63}Cu(n,\gamma)^{64}Cu$

$^{54}Fe(n,p)^{54}Mn$

$^{58}Ni(n,p)^{58}Co$

sono state utilizzate sottili targhette di materiale ultra puro e posizionate contemporaneamente nelle tre diverse posizioni. La scelta di tali reazioni è dovuta al fatto che mediante tali reazioni nucleari è possibile ricostruire con sufficiente precisione lo spettro neutronico nel range energetico 0 – 18 MeV. Tuttavia, il set di targhette sottili è in grado solo di essere utilizzato per determinate la componente termica e veloce dello spettro. Per rendere sensibili le targhette alla componente epitermica si è dovuto ricorrere alla copertura delle targhette mediante sottile foglio di cadmio. In questo modo, avendo il cadmio una elevata sezione di cattura neutronica per neutroni termici, le targhette di oro e

rame assorbono principalmente la parte epitermica e veloce del flusso. In alcuni irraggiamenti si è quindi utilizzato il cadmio per tagliare la parte termica.

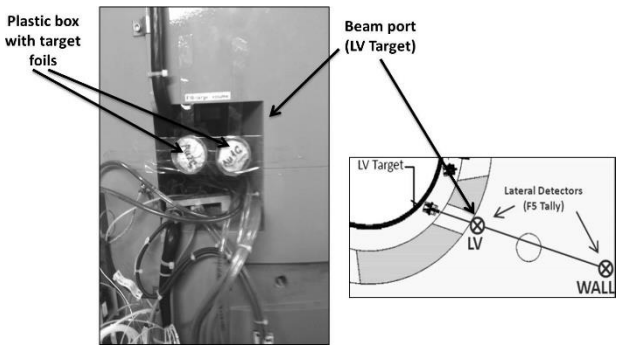

Figura 7: Particolare del posizionamento delle targhette nella posizione LV. A destra lo schema rappresentato dal modello geometrico.

L'irraggiamento di ogni set di targhette (ad una intensità di fascio di 30 μA) è stato condotto per 60 minuti in modo tale da generare nelle targhette, attraverso il campo neutronico secondario, un'attività indotta sufficiente da essere misurata. A fine irraggiamento le targhette sono state rimosse dalla loro posizione (Figura 7) e l'attività indotta è stata misurata con un detector al germanio ultra puro (HPGe) e analizzata con il software GammaVision ®.

Risultati

I valori calcolati delle attività indotte dalle diverse reazioni nucleari sono state utilizzate come input per il programma SAND II, il quale processa le differenti misure sperimentali relative alle singole attività specifiche a saturazione. Le attività specifiche a saturazione sono state calcolate a partire dalle attività indotte nelle targhette sottili considerando le varie correzioni dovute a processi di decadimento, tempi di conteggio e attesa. Ulteriori dettagli sulla procedura utilizzata si possono trovare in [12].

Il confronto tra gli spettri Monte Carlo e gli spettri sperimentali riprocessati dal codice SAND II sono presentati nelle Figure 8, 9 e 10 per le tre differenti posizioni LV, WALL e TOP.

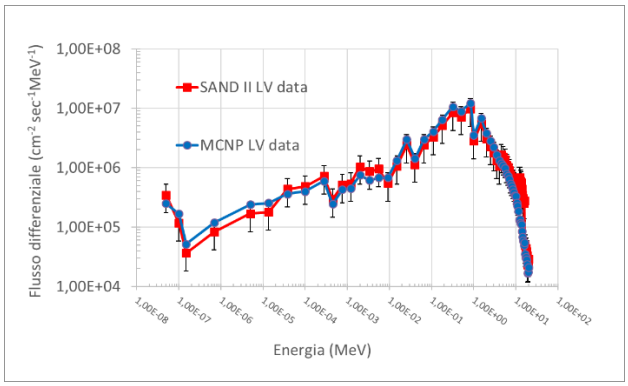

Figura 8: Confronto tra i dati di MCNP6 e le misure sperimentali elaborate da SAND II per la posizione LV.

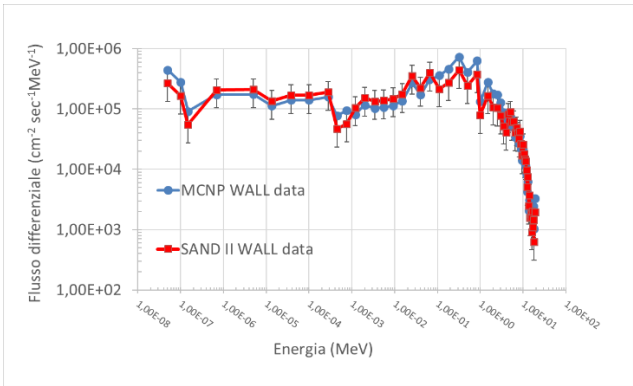

Figura 9: Confronto tra i dati di MCNP6 e le misure sperimentali elaborate da SAND II per la posizione WALL.

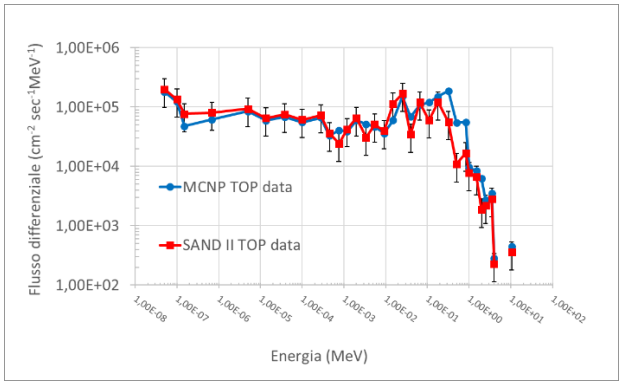

Figura 10: Confronto tra i dati di MCNP6 e le misure sperimentali elaborate da SAND II per la posizione TOP.

Il valore dei flussi totali e termici per le tre differenti posizioni è riportato nella Figura 11. Il flusso termico è stato calcolato sommando i bin energetici da 0 a $6.9 \cdot 10^{-7}$ MeV.

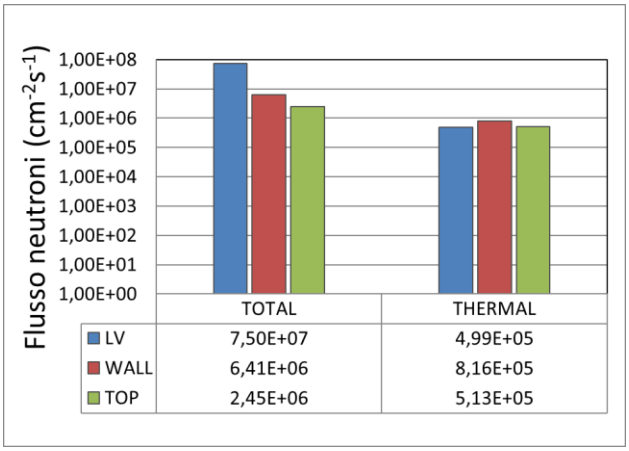

Figura 11: Confronto tra spetto totale e parte termica per le tre diverse posizioni riportate.

A causa di eventi di scattering, gli spettri sono traslati verso energie minori rispetto allo spettro sorgente e tale traslazione è più evidente nelle posizioni TOP e WALL piuttosto che LV. Questo avviene perché i neutroni sono soggetti a più collisioni man mano che la loro distanza dalla sorgente (localizzata nel target) aumenta, inoltre lo spettro è molto più termalizzato nelle vicinanze della parete del bunker a causa dei materiali a basso Z di cui è costituita la parete stessa. I grafici calcolati e misurati, riportati nelle Figure 8, 9 e 10, mostrano lo stesso andamento e sono in buon

accordo. Inoltre gli spettri ottenuti nella posizione più vicina al target mostrano un picco nella regione a più alte energie che è traslato ad energie minori man mano che la distanza dal punto di misura al target aumenta, zona in cui il contributo dei neutroni termici ed epitermici risulta essere maggiore.

Stima della dose ambientale H(10)*

Gli spettri ottenuti con MCNP6, tenuto in considerazione i coefficienti di conversione dose ambientale-fluenza [13] sono stati utilizzati per la stima della dose ambientale H*(10) nelle posizioni LV, WALL e TOP. I valori ottenuti sono riportati in Tabella 1.

position	H*(10) (mSv/μA.h)
LV	56
WALL	84
TOP	27

Tabella 1: Valori di dose ambientale H*(10) in mSv/μA.h ottenuti con MCNP6 nelle tre diverse posizioni (l'errore associato a ogni valore è del 5%).

Il valore maggiore per H*(10) si osserva nella posizione WALL, mentre nelle posizioni LV e TOP il valore di H*(10) risulta inferiore a causa della modifica dello spettro (spostamento verso energie più basse) prodotta dai materiali costituenti la struttura del ciclotrone e dallo scattering nelle vicinanze della parete in cemento del bunker.

Conclusioni

Durante il bombardamento dell'acqua arricchita in ^{18o} per la produzione di ^{18}F si genera un campo secondario di neutroni dalla reazione (p,n) che dà luogo a specifici requisiti di radioprotezione. La conoscenza dello spettro neutronico consente, in previsione di un futuro decommissioning dell'acceleratore, di poter fare previsioni sulle evoluzioni temporali delle concentrazioni dei vari radionuclidi prodotti nei materiali che costituiscono il ciclotrone e nel calcestruzzo del bunker che lo ospita.

I risultati presentati forniscono una caratterizzazione del campo neutronico soddisfacente sia dal punto di vista modellistico che sperimentale nonché una validazione del metodo utilizzato già in un lavoro precedente [12].

Si sottolinea inoltre che il metodo sperimentale utilizzato può essere applicato con successo solo in presenza di campi neutronici sufficientemente intensi da indurre un'attività nei materiali che costituiscono le targhette sottili che sia misurabile ed inoltre la tecnica deve essere ottimizzata in ogni specifico caso (ad es. la selezione delle targhette di materiale differente). In generale tale ottimizzazione risiede nella corretta selezione dei materiali da irraggiare caratterizzati da particolari sezioni di assorbimento neutronico e soprattutto in grado di ricoprire il range energetico di interesse relativamente allo spettro neutronico.

La ricostruzione della geometria e dei materiali dei target consentirà in futuro di poter estendere questo studio alla dinamica del target in termini di attivazione dei materiali. Tali informazioni risultano molto utili per i programmi di gestione della macchina in particolare nelle fasi

di manutenzione ordinaria e straordinaria in cui sono previste principalmente manipolazioni di parti molto attivate quali i target.

Ringraziamenti

Si coglie l'occasione di ringraziare i colleghi di IBA Belgio per la continua collaborazione e scambio di informazioni tecniche e scientifiche.

Referenze

[1] Alloni, D.; Prata, M. Characterisation of secondary neutron field generated by a compact PET cyclotron with MCNP6 and experimental measurements. *Applied Radiation and Isotopes* **128** 204–209 (2017)

[2] Briesmeister, J.F. MCNP – A General Monte Carlo N-Particle Transport Code, Version 4C, *Los Alamos National Laboratory Report LA-13709-M* (2000)

[3] Alfassi, Z.B. Chemical Analysis by Nuclear Methods. *John Wiley and Sons, Inc*. (1994)

[4] Bertini, H.W. Low energy intranuclear cascade calculations. *Phys. Rev.* **131** (4), 1801 (1963)

[5] Bertini, H.W. Low energy intranuclear cascade calculations. *Phys. Rev.* **138**, AB2 (1965)

[6] Carroll, L.R. Radiation measurements related to the design of a self-shielded accelerator system for routine use in PET. *In: Proceedings of the 34th Annual Meeting of the Society of Nuclear Medicine*, vol 28 (1987)

[7] Carroll, L.R. Predicting long-lived induced activation of concrete in a cyclotron vault. *In: AIP Conference Proceedings* **576**, 301–304 (2001)

[8] Carroll, L.R. Estimating the radiation source term for PET isotope targets. *In: Proceedings of the 9th International Workshop on Targetry and Target Chemistry* (Turku, Finland) (2002)

[9] Berg, S.; McElroy, W.N. A Computer-automated Iterative Method for Neutron Flux Spectra Determination by Foil Activation, Vol. II: SAND II (Spectrum Analysis by Neutron Detectors II) and Associated Codes, AFWL-TR-67-41 (1967)

[10] Griffin, P.J. SNL RML Recommended Dosimetry Cross Section Compendium SAND-92-0094 (1993)

[11] Griffin, P.J. User's Manual for SNL-SAND-II Code SAND-93-3957 (1994)

[12] Alloni, D.; Borio di Tigliole, A.; Bruni, J.; Cagnazzo, M.; Cremonesi, R.; Magrotti, G.; Oddone, M.; Panza, F.; Prata, M.; Salvini, A. Neutron flux characterization of the SM1 sub-critical multiplying complex of the Pavia University. *Prog. Nucl. Energy* **67**, 98–103 (2013)

[13] ICRP 119. Compendium of dose coefficient based on ICRP publication 60. Ann. ICRP 41 (Suppl 1) (2012)

EPIGENETICS, EVOLUTION AND IONISING RADIATION
Part B: EPIGENETICS AND IONISING RADIATION

Mauro Belli

mau.belli1@gmail.com

1. Ionising radiation as a genotoxic agent: implication in health risk assessment

Ionising radiation is a well known genotoxic agent, capable of inducing a wide spectrum of DNA alterations, such as: base damage, sugar damage, single strand breaks (SSBs), double strand breaks (DSBs), DNA-DNA and DNA-protein cross links. Clustered DNA damage[1] is the most biologically relevant radiation-induced DNA damage [1,2,3,4,5] as it is expected to be less readily repaired as compared to most cellular-induced damages. Also at low doses the relevant damage is caused by the passage of single particles that can produce clustered DNA lesions [2,4,6], such as complex DSBs and non-DSB clustered lesions [7].

Damage complexity increases with linear energy transfer (LET) of radiation. High LET charged particles characteristically induce complex chromosome aberrations[2] [8,9] as defined by Savage and Simpson [10], even if they can also be observed after exposure to γ-rays. In particular, high-LET heavy-ions induce a high fraction of complex-type exchanges, and possibly unique chromosome rearrangements [11,12].

It is generally assumed that a vast majority of mutations are neutral or detrimental[3], as in many circumstances gene mutation is a process which acts contrary to natural selection and which burdens each population with a load of harmful genes [13]. On the other hand beneficial mutations may occur that, despite their rarity, cause long-term adaptation. However, considering the low likelihood of beneficial mutations, radiation induced mutations in humans are generally assumed to increase both the probability of developing cancers and the rates of hereditary diseases that occur naturally in the population. It is not surprising, then, that most studies about radiation-induced mutations are focussed on radiation protection issues and that relevant reviews and evaluations have been conducted by several International and National bodies devoted to this matter, such as UNSCEAR[4], ICRP[5], and BEIR[6].

2. The conventional paradigm of Radiobiology: epigenetics-related issues

[1] DNA clustered lesions consist of multiple DNA lesions (either strand breaks or other lesions) in close proximity. More exactly clustered damaged sites are defined as two or more lesions formed within one or two helical turns of DNA by a single radiation track [2,4]. A complex DSB is a DSB with additional lesions close to it. They can be classified according to their "complexity". The proportion of complex DSB and the degree of complexity increase with increasing radiation [14].

[2] Complex chromosome aberrations are defined as those aberrations involving three or more breaks in two or more chromosomes [10].

[3] In 1926, Muller discovered that, by exposing the fruit fly *D.melanogaster* to high levels of radiation (such as X-rays or gamma rays from radioactive materials), the mutation rate in their offspring can be increased by as much as 150 times. (see ref [15] for a review). For this discovery he was awarded the 1946 Nobel Prize in medicine and physiology. His work convinced him that the vast majority of mutations were deleterious and consequently that exposure to radiation should be strictly controlled [16].

[4] UNSCEAR, United Nations Scientific Committee on the Effects of Atomic Radiation

[5] ICRP, International Commission on Radiological Protection

[6] BEIR, Biological Effects of Ionizing Radiation, National Research Council, National Academy of Sciences, U.S.A.

After more than a century of research aimed at underlying the radiobiology bases of detrimental effects of ionising radiation, the following overall picture has been developed, sometimes called "conventional paradigm of radiobiology" [17]:

i) The DNA damage in directly exposed cells is the main event for biological effects

ii) DNA damage occurs during, or very shortly after, irradiation of the nuclei in targeted cells

iii) The potential for biological consequences can be expressed within one or two cell generations

iv) At low doses the biological effect is in direct proportion to the energy deposited in nuclear DNA (this is the rational basis for assuming a Linear No-Threshold (LNT) relationship between risk and dose).

The Internationally agreed system for radiation protection has developed from this paradigm, although with many simplifications and assumptions [18]. This paradigm, based on the target theory of radiation-induced effects, has been challenged since the beginning of the '1990s, from the results of *in vitro* studies that demonstrated the occurrence of the so-called "non-targeted effects" (NTE), i.e., in cells not directly traversed by radiation. Of particular interest appear those not represented by the damage in DNA of the irradiated cells, such as the "bystander effect"[7] (BE) [19], reviewed in [20] and delayed radiation effects, i.e., genomic instability[8] (GI) [21,22]. These phenomena have been seen in many in-vitro and in-vivo experiments, including mouse experiments, and in experiments with blood samples from irradiated humans. [22,23].

Another phenomenon that does not fit into the conventional paradigm is the radiation-induced adaptive response[9] (AR) discovered even earlier [24], and often included in the category of non-targeted effects. All these phenomena are interpreted as manifestation of general phenomena, such as intra- or inter-cellular signalling and can represent different and even opposite behaviour in the cell response to ionising radiation, giving rise to non linear responses.

Since then numerous *in vitro* and *in vivo* studies have been performed and their relevance for radiation risk assessment is being evaluated [25,26,23], and the question can be raised whether a new paradigm has to be developed [17,27].

It is clear that, in order to answer this question, a substantial improvement is required of our understanding of the biological consequences of exposure to ionising radiation, especially those at low dose that is the kind of exposure typically encountered in the workplace, in the environment and in diagnostic medicine. The importance of research aimed to reduce low-dose risk uncertainties has recently been recognised at International and European level [28,29].

Such a development also requires revisiting some critical concepts used in radiobiological research, taking into account the following observations.

a) Cells do not respond independently. This is the problem of how the target is defined. If cells respond independently, then the target should be a cell; but in general in an organism the tissue response is important. Even in a cell population cultivated in vitro the available data suggest that the tissue function is more complex than the sum of its cellular parts. Phenomena such as the bystander effect have shown that integration by extracellular signalling through the microenvironment must be considered [30]. In

[7] A commonly accepted definition of "bystander effect" is that proposed by Djordjevic [31] according to which a bystander effect describes the ability of cells affected by an agent to convey manifestations of damage to other cells not directly targeted by the agent or not necessarily susceptible to it *per se*. Thus radiation induced bystander effects are effects manifesting in cells that were non-irradiated, neighbours of irradiated cells or that received factors secreted or shed by irradiated cells.

[8] Genomic instability is an all-embracing term to describe the increased rate of acquisition of alterations in the genome. It is measured, e.g., as chromosomal alterations, changes in ploidy, micronucleus formation, gene mutations and amplifications, etc. Radiation-induced genomic instability manifests in the progeny of exposed cells [21].

[9] Adaptive response (AR) to radiation is a transient phenomenon that has been observed in cells, tissues and organisms when a small conditioning radiation dose (called 'priming dose') reduces the biological effects of a subsequent (usually higher) radiation dose (called 'challenging dose'). Early evidence of this effect was shown in human lymphocytes

general, the cellular system responds as a whole and therefore, especially at low dose, the cell population must be seen as a single entity perturbed by radiation, and the response comes from the whole population in a coordinated way [32]. This consideration calls for a carefully evaluation of the meaningfulness of cell cultures as biological models, as in many instances they cannot be seen as sets of autonomous cells.

b) Involvement of non-DNA targets. A second issue comes from the observation that radiation and many other genotoxic agents do not only damage DNA, but they also damage other cellular components capable of influencing the functioning of the cell. Among these "complementary" targets, consideration should be given to the cellular defence systems. An important class of these targets is represented by the DNA repair system and cell cycle checkpoint pathways, closely interacting to maintain genetic integrity in the events of DNA damage [33]. They are part of the highly complex signalling response termed the DNA damage response (DDR) that is used by the cell to counteract the deleterious effects of DNA damage [34,35,36].

3. Ionising radiation induces epigenetic changes

Even if it is usually thought that ionising radiation acts through DNA damage, it may also cause epigenetic[10] alteration. In effect, epigenetic events are known to regulate gene activity and expression not only during development and differentiation, but also in response of environmental stimuli, including ionising radiation (see the reviews in [37,38,39])**.**

Genetic mechanisms, such as mutations, are heritable, but not very susceptible to environmental influence. Indeed, a mutation is a relatively rare event. On the contrary, metabolic pathways are responsive to environmental changes through interactions of chemicals or other stressors with proteins involved in gene expression, but are not heritable. Epigenetic modifications are instead extremely susceptible to environmental change and heritable at the same time. They can persist after the stressor is removed, but they can also be reversible [40]. DNA methylation, the first studied epigenetic modification, presents both similarities and differences between plants and animals [41].

Many observations have been reported in the literature, showing or suggesting an epigenetic role in radiation-induced biological effects. Epigenetic events are likely involved in both aspects a) and b). For example they may have a role in cell-cell communication and may also alter the DNA repair pathways. A well known epigenetic event is the modifications in DNA methylation that regulate gene expression through chromatin structure modification (see part A of this paper), and this may also affect expression of repair enzymes.

Early findings obtained in the '80s have indicated that exposure to X- or γ-rays can change the DNA methylation pattern both *in vitro* and *in vivo* (see the review in [42]). It has been demonstrated that in cultured human cells irradiation results in the induction of heritable methylation changes [43,44]. Rodent models have shown that DNA methylation changes are associated with radiation-induced changes in a dose-dependent, sex, and tissue-specific manner [45,46]. Modifications in DNA methylation levels have been found in adult mice irradiated with high doses of low-LET radiation and, interestingly, these changes were not ubiquitous among different tissues and cells [47].

Other mechanisms, besides DNA methylation changes, capable of inducing modifications in chromatin structure, act through histone modifications. For years histones were regarded as merely structural proteins but now they are recognised to play an important role in regulating gene expression [48]. They can undergo various modifications such as acetylation, methylation, phosphorylation, ubiquitination, which change gene transcription by changing DNA accessibility but also by recruiting other proteins that act to alter chromatin structure actively or to promote transcription.

[10] The modern definition of Epigenetics is "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence". The epigenetic trait of an organism is intended as the "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" [49]. See also Part A of this article published in a previous issue of this journal.

A well-known radiation-induced histone modification is phosphorylation of histone H2AX, which is crucially important for the repair of DNA strand breaks and for the maintenance of genome stability. Furthermore, it has been shown that chromatin modification by histone acetylation is also crucial for DNA repair [50], and that chromatin acetylation is involved in several important steps such as chromatin remodelling and tagging of DSBs, activation of repair regulators, cell cycle regulation, and apoptosis. [51].

Another type of epigenetic radiation-induced modification involves non-codingRNAs (ncRNAs), in particular microRNAs (miRNAs) that have a role in the regulation of gene expression. MiRNAs are involved in the response of irradiated cultural human cells [44].Since their discovery in 1993 [11] miRNA are emerging as important modulators in many cellular pathways, including cell radiosensitivity and radioresistance. They are estimated to regulate the expression of up to 60% of the human protein coding genes [52,91] by means of mRNA degradation or translational repression acting through a multitude of interconnecting regulatory pathways [53,54,55,56,57].

The roles of specific miRNAs have only recently begun to be elucidated. A number of studies have examined the general and specific effects of miRNAs perturbation in different cell types exposed to photon ionising radiation, i.e., X- or γ-rays (see the review of ref [58]). These studies revealed that the expression levels of several miRNAs change significantly upon irradiation and indicated a specific role of various miRNAs on cellular radiosensitivity [59].

4. Radiation quality affects epigenetic changes

Most research on the impact of radiation exposure on the epigenome has focused on the effects of low-LET (X- or γ-rays). Many of these studies report global hypomethylation in response to relatively high doses of X-rays (e.g., up to 10 Gy) [42,44,45,60], an effect interpreted as a consequence of the difficulty of the "maintenance" DNA methyltransferase (DNMT1) to take care of the newly synthesized DNA generated during the repair process [61], or due to a reduced expression of DNA methyltransferases in exposed animals [46].

In contrast, few studies have assessed the effects of high-LET radiation on the epigenome. It is expected that, given the unique characteristics of the high-LET radiation track and the consequent damage, there is the potential for unique effects on the epigenome. Indeed, some findings indicate that exposure to high-LET radiation can result in lasting changes in the total levels of DNA methylation and in the miRNA expression that may be different from those induced by equivalent doses of low-LET radiation [42,44,62,63,64] (see also the review of ref.[65]). It is now widely accepted that epigenetic aberrations induced by low linear energy transfer (LET) irradiation are different than those induced by high LET irradiations, but little information is available on the effect of high-LET radiation at specific genes.

Some study have been focussed on the effect of high energy and charge (HZE) nuclei, the most detrimental component of space radiation associated to health risks encountered by astronauts in deep space [66,67]. Comparison between X-ray and high-LET Fe-ions exposures of cultured cells showed that, although Fe-ions elicited more chromosomal damage and cell killing, a lower incidence of epigenetic changes (in terms of alteration of miRNA expression levels) was observed after exposure to X-rays [44]. Also global DNA methylation could be different, as hypermethylation was found in cells exposed to protons and high-LET Fe-ions in contrast to hypomethylation for cells exposed to X-rays [42,60,64]

A possible explanation for this difference comes from the observations that stable DNA methylation can result at the sites of DNA break repair [70] that is likely produced with higher yield by high-LET radiation.

---

[11] The first microRNA (miRNAs) was discovered in 1993 (Lee et al 1993) [68]. MiRNAs are endogenous small (20-30 nucleotide long) non-coding RNAs that can play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression (Carthew and .Sontheime 2009) [69].

Changes in miRNA expression induced by irradiation with high energy protons, γ-rays, or ^{56}Fe-ions in mouse blood showed a radiation type- and dose-specific response pointing to a specificity of the damage induced by the different types of radiation, although there were some overlapping in the target genes [71,72].

5. Basic mechanisms

There is evidence for a role of reactive oxygen species (ROS) in epigenetic processes through the generation of oxidative stress[12] [73,74,75], so that a plausible mechanism for initiating and perpetuating genome instability caused by ionising radiation is provided by the induction of oxidative stress similarly to that assumed for other metabolic, dietary, or environmental agents [76]. Indeed, persistently increased levels of intracellular reactive oxygen species (ROS) have been found in chromosomally unstable cells [25]. Oxidative stress can cause a wide range of DNA lesions that include base modifications, deletions, strand breakage, and chromosomal rearrangements leading to genetic as well as epigenetic alterations [77].

Oxidative DNA damage can modify the epigenome by multiple mechanisms. One important form of damage is oxidation of DNA bases, with the main target being the CpGs (especially in the CpG islands). Oxidation of guanine to 8-hydroxy-2′-deoxyguanosine (8-OHdG) can interfere with the ability of DNA to function as a substrate for the DNA methyltransferases (DNMTs), inhibiting DNA methylation at nearby cytosine bases, while oxidation of cytosine to 5-hydroxymethylcytosine (5hmC), may achieve active DNA demethylation. These processes lead to loss of the inhibition of the binding of transcription factors within otherwise properly methylated CpG islands with loss of the epigenetic regulation [78] and may result in potentially heritable epigenetic alterations [76]. This mechanism is responsible for inducing global DNA hypomethylation and subsequent genomic instability [77]. ROS can also function as catalysts of DNA methylation, so that oxidative damage can influence both aspects of DNA methylation changes (hypo- and hyper-methylation) through different mechanisms, which play an important role in epigenetic regulation in cancer cells [79].

Mitochondria can also have a role in radiation-induced global DNA hypomethylation as high intra-mitochondrial ROS level can damage the mitochondrial DNA and its mutations affect the epigenetic control mechanisms of the nuclear DNA, by decreasing the activity of methyltransferases. These changes can be transmitted to the progeny of the irradiated cells [80].

Changes in DNA methylation are not isolated events, as sometimes they are accompanied by histone modifications. These changes closely interact in setting the transcriptional states of chromatin. [81]. Histones act as transcriptional regulators by modulating the accessibility of DNA functional regions through a number of post-translational modifications. They predominantly occur at the N-terminal tails protruding from nucleosomes, that is capable to alter the spatial structure of the chromatin and associate specific proteins leading to an increase or decrease of transcription [82]. These modifications include acetylation, methylation, phosphorylation and ubiquitination. Different modifications at several amino acids at different histone tails are possible, and there is interdependence between them [83]. For example, histone acetylation is linked to transcriptional activation, since acetylated histone tails lose their positive charge, reducing their affinity for the negatively charged DNA, and leading to a relaxed chromatin packaging. In contrast, histone deacetylation is an opposite repression event. These modifications are thought to constitute an epigenetic ‘histone code” [82]. Methylation of a particular amino acid (lysine 27) in histone H3 is found to repress genes, probably because it is a prerequisite for subsequent DNA methylation. [84].

Changes in chromatin organization in the direct vicinity of DSB induced by ionising irradiation appear to be dynamic and related to modifications of histones such as H2AX that has a well-known role in the response of radiation-induced DSBs. They result in chromatin relaxation around the DSB immediately

[12] Oxidative stress is the result of an imbalance between reactive oxygen species production and the defense (antioxidant) mechanisms.

after exposure but, within typically 40 min, these changes are replaced by increased methylation at H3K9, leading to the condensed state of the chromatin [85,86].

Also expression of ncRNAs, in particular that of miRNAs, interact with DNA methylation. It has become clear that microRNAs play important roles in governing DNA methyltransferases (DNMTs) which are enzymes responsible for setting up and maintaining DNA methylation patterns at specific regions of the genome. Some miRNAs targeting DNMT transcripts lead to the demethylation and transcriptional activation of numerous protein coding gene sequences, thereby contributing to gene expression [87]. In turn, DNA methylation and histone modifications can affect the expressions of miRNAs [88], revealing a feedback loop between miRNAs and DNMTs [89, 87].

Another example of the interplay between miRNA and chromatin organization comes from the observation that in hematopoietic cell lines a specific miRNA controls the histone variant H2AX [90]. The importance of mechanisms associated to miRNAs relies on the observation that they regulate the expression of up to 60% of protein-coding genes [91,92].

It is important to note that research on radiation-induced epigenetic mechanisms was initially addressed to DNA methylation as a process capable of modulating gene expression by changing chromatin organization, and subsequently integrated with the roles of histone modifications and changes in miRNA expression as they would act independently. However, recent findings, such as the observation that DNA methylation and histone methylation are tied together in a reinforcing loop [93], speak in favour of a strong interplay between DNA methylation, histone modification and miRNA expression.

6. Epigenetic mechanisms have a role in non-targeted effects (NTE) of ionising radiation

In the last two decades a wealth of investigations have been carried out on "non-targeted effects" (NTE), i.e., on biological changes observed in cells not directly traversed by radiation, notably bystander effects (BE), genome instability (GI, which in turn is related to transgenerational effects) and adaptive response (AR). These phenomena challenge the concepts on which the conventional paradigm of radiation biology is based and are potentially relevant for radiation risk assessment [23].

There is evidence that these phenomena are inter-related and that they may share some common mechanistic pathways (see, e.g., [94,95]), with AR mechanisms perhaps overlapping less to those of BE and GI [23], so that it is not always possible to make a clear distinction between them. For example the radiation-induced intercellular signalling cascades, including cytokine production, nitric oxide production and persistent free radicals have the potential to mediate both instability and bystander effects. Interestingly, GI was observed in progeny of unirradiated neighbours of irradiated cells since 1998 [94].

In vitro experiments have provided some important insights into the nature of these effects, but their mechanisms remain to be completely understood, in spite of extensive research. An intriguing observation is that, even if NTE have been observed in a variety of cell and tissue types, and for a variety of radiation qualities, they have not been universally observed [96,97,23,98,99].

Most NTE have been observed *in vitro*, but they can also be relevant *in vivo*, even if the question remains whether the non-targeted effects demonstrated in vitro can be extrapolated to in vivo situations. BE was found in rodents since exposure to one side of the animal body caused profound changes in the unexposed portion of the body [100]. Another important observation was that the induction of malignancy in the shielded head (specifically in the brain) of radio-sensitive mice by exposure of the remainder of the body to X-rays occurred at a frequency much higher than that which would be induced by the scatter dose to the head [101]. These observations could be relevant to the understanding of the mechanisms of radiation carcinogenesis and of the abscopal effect in radiotherapy[13].

[13] The term "abscopal effects" is used especially in radiotherapy to describe the effects occurring outside the radiation fields in patients. In these effects systemic responses may play a role.

Epigenetic mechanisms, encompassing DNA methylation, histone modification, and RNA-associated gene silencing, have been shown to be plausible mediators of the indirect radiation effects. These inter-relationships have stimulated much interest especially for their possible impact in the risk assessment at low radiation doses and have been the subject of a number of interesting reviews [102,103,104,105,98].

There are many lines of evidence that epigenetic mechanisms have a potential role in genetic instability. An early *in vitro* observation was made on micronuclei induction in cultured cells irradiated with different fluences of alpha-particles, indicating that the target for genomic instability is larger than the cell nucleus [106], which gives an indirect support to the involvement of epigenetic mechanisms, possibly via BE between hit and non-hit cells.

Genome instability is observed in a much larger proportion of cells irradiated with low to medium doses than for mutations from targeted effects (reviewed in [23]) suggesting that instability might arise, rather than from a genetic mutation, through epigenetic mechanisms [107, 102]. Animal-based studies demonstrated that exposure to ionising radiation alters epigenetic parameters in the directly exposed tissues but also in the distant bystander tissues and that these changes are still observed seven months after exposure [100].

Experimental evidence points to a link between genome instability in the exposed animals and the radiation-induced global DNA hypomethylation (see the review in [105]). The fundamental role of DNA methylation in the transmission of GI is clearly demonstrated in embryonic stem cells since the elimination of DNA methylation genes completely eliminates transmission of GI. Interestingly, this inactivation also clears the memory of the radiation insult, protecting neighbouring cells from indirect induction of genomic instability [108]. There is evidence in eukaryotic cells that, besides DNA methylation, also miRNA have a role in radiation-induced GI and BE [86].

It has to be noted that association of radiation-induced genomic instability with heritable DNA methylation changes has been shown in cultured human cells at high doses of radiation (>1 Gy) [109,108,] so that their extrapolation to in vivo situations of interest for radiation protection is highly questionable. A review published by UNSCEAR [110] suggests a likely threshold for the induction of transmissible instability of 0.5 Gy for low-LET radiation, and a generally, higher effectiveness for high-LET radiation.

Concerning the BE, it was shown that miRNAs are also involved in radiation-induced bystander responses, but these investigations are not much developed (see the review in [99]). Experiments conducted *in vivo* have shown that exposure to one side of a mouse body induced a significant upregulation of a specific miRNA in distant lead-shielded liver tissue, which was suggested to cause downregulation of two proteins responsible for DNA methylation in the same bystander tissue [100]. Moreover, upregulation of miRNAs has been found in directly exposed male mice, leading to hypomethylation of the exposed animals as well as of their unexposed offspring, demonstrating the possibility that they may play a role in transgenerational epigenetic inheritance of genomic instability [111].

Information about the possible role of epigenetic mechanisms in the adaptive response to ionising radiation is scarce. AR can be regarded as a quite general phenomenon as it has been observed in cells, tissues and organisms using various indicators of biological damage as a consequence of exposure to ionising radiation and also to other stress agents. AR is relevant for the biological response at low doses/dose rates. Although in the literature there is a plethora of descriptions about the adapting conditions, the mechanisms underlying radioadaptive responses remain poorly understood [112,23,113].

Both intracellular and intercellular signalling (the latter being related to BE) can account for the occurrence of radioadaptive responses. The main proposed mechanisms to explain AR rely on increased efficiency of DNA repair and induction of anti-oxidant enzymes, both of them being used to model AR [114]. Enhanced efficiency of DSB repair through homologous recombination and also a significant increase in gene expression of antioxidant enzymes appear to play a predominant role in the adaptive response (see the review in [27]). It has been proposed that, in order to adapting the gene expression

programme to the stress situation, and to proper functioning of DNA repair processes, epigenetic processes are involved, notably transient protein acetylation [51].

7. Epigenetic memory and radiation induced transgenerational epigenetic effects

The heritable change in gene expression that is induced by a previous stimulus, such as exposure to ionising radiation, is often described as epigenetic memory[14].

Environmental stressors, in particular, can cause this sort of "footprint" on the epigenome that, under specific conditions, can be transgenerational. Epigenetic variation induced by environmental factors contributes to the phenotypic plasticity and adaptive capacity of various species. The molecular basis of cellular memory is a fascinating topic that as been addressed during the last few decades [115].

DNA methylation is historically the first epigenetic modification discovered in mammals. In mammals, DNA methylation is typically removed during zygote formation and then re-established in the embryo through successive cell divisions during development. Methylation modifications that regulate gene expression are usually heritable through mitotic cell division; some methylation is also heritable through the specialized meiotic cell division that creates egg and sperm cells, resulting in genomic imprinting**.** Therefore, although DNA methylation is dynamic, some DNA methylation patterns may be retained as a form of epigenetic memory [115]. The presence of specific DNA methyltransferases, as "maintenance" enzymes, ensures that established methylation patterns can be faithfully copied so that DNA methylation can template its own inheritance [116]. In contrast, most histone modifications are not heritable and the extent to which a histone moiety can template its own inheritance is still a matter of debate and investigation [116,117]. Among the few examples of histone "inheritance", there is the methylation of histone H3 that is found to be epigenetically transmitted during development and across generations [118]

Some epigenetic changes have been proven to be stable and can lead to transgenerational[15] heritable changes. In plants and in some animals such as nematodes, transgenerational epigenetic inheritance is well-documented and relatively common [119] as a response to the environment, including ionising radiation. Radiation-induced transgenerational effects may involve radiation-induced genome instability. Indeed, *in vitro* data have shown that ionising radiation can induce genomic instability that can manifest in the progeny of the irradiated cells for many divisions [22]. The observed transgenerational gene expression phenotype was also termed ''epigenomic instability'' [120].

The first evidence for a radiation-induced transgenerational effect was reported in 1976 by Luning et al. [121], who showed elevated rates of dominant lethal mutations following intraperitonial injection of male mice with a plutonium salt solution. Afterwards, animal models have demonstrated that effects of the parental radiation exposure are transmitted through the germline to the progeny of the irradiated parent [122, 21,22,123] and the analysis of mutation induction has provided evidence for an elevated germline mutation rate in the parents directly exposed to ionizing radiation and a number of chemical mutagens, resulting in various transgenerational effects [124] (see also the reviews in [125,126]). Radiation-induced cancer predisposition was also reported [122] although the general validity of the conclusions for this endpoint is questioned [127]. Literature on the risk or diseases in the offspring after maternal exposures to ionizing radiation is growing, but also transgenerational epigenetic effects from paternal exposures have been observed in animal models suggesting that the radiation exposure signal could be inherited through epigenetic changes in paternal sperm [128, 125,129,130].

---

[14] Epigenetic memory can be considered over different time scales: *cellular and transcriptional memory* (mitotically heritable) and *transgenerational memory* (meiotically heritable). (see, e.g., the review in [117]).

[15] In organisms a transgenerational transmission of phenotypes is different from intergenerational transmission. It occurs when the effects of the parental exposure are present also in the first generation that is not directly exposed. Since in general parental exposure can also affect their germ lines, this first generation is not the F1 but is the F2 generation, and is the F3 generation if eposure occurs during pregnancy. (se, e.g., [142]). In this paper "transgenerational epigenetic effects" are intended as those effects which arise in the offspring of the irradiated organism and that are not due to the inheritance of DNA mutations through the parental germline (see Little et al 2013 for more details).

Although the exact molecular mechanisms of transgenerational genome instability are not completely elucidated, there is support for a role of epigenetically induced phenomenon with a persistence for multiple generations of the radiation-induced cellular reprogramming [22,131, 125, 132, 133]. The conclusion of an epigenetic origin of radiation-induced genomic instability in the offspring is based on the two observations that radiation-induced genomic instability persists over a long period of time after the exposure and that the number of cells/organisms with radiation-induced genomic instability is too high to be explained by direct targeting of genes. [134,135].

Almost all the data in this field concern biological systems exposed to low-LET radiation (X- or γ-rays), but some data are now emerging on the effects of radiation of different quality. It has recently been reported on transgenerational effects on *C. elegans* reproduction caused by proton beam irradiation. [136]. A sex-dependent inheritance of DNA methylation (the classic epigenetic mark) was reported as a biological response to the exposure of rats to low doses of uranium [137,138,139].

A review on long-term effects of ionising radiation on a number of plant and animal species observed in Chernobyl, conducted in the framework of the EU funded COMET Project, indicates that in several cases there is no direct evidence to link morphological or reproductive effects to genetic changes, suggesting a highly probable role of epigenetic mechanisms. [140]. This project also gave the first indications that DNA methylation might be affected over several generations in different organisms both in the lab and in the field. [141].

The consequence in terms of health risks to the progeny of irradiated parents due to transgenerational genomic instability has been studied in animal models and in humans (see the reviews in [143, 102]). The occurrence of genomic instability in succeeding cell generations has been suggested in children of irradiated parents in the Chernobyl accident [144]. However, in contrast to animal data, only a limited number of human data exist concerning the risk in the offspring of parents exposed prior to conception so that the hypothesis of transgenerational induction of increased cancer susceptibility after paternal radiation exposure in humans has long been controversial [145, 127].

8. Epigenetic effects are involved in radiation-induced cancer

There is large consensus on the fact that cancer is a disease that results from both genetic and epigenetic changes and several studies point to the description of cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host, with mechanisms involving both genetic mutations and epigenetic modifications [146,147,148,81]. This notion applies not only to solid cancers but also to leukaemia, in particular to myeloid leukaemia [149]. Dramatic changes in DNA methylation are common in cancer and are considered as an early event in many of them [150,151,152]. Indeed, DNA methylation changes appear to be more frequent events than genetic mutations [153,154].

The global loss of DNA methylation at CpG dinucleotides was the first epigenetic abnormality identified in cancer cells [155]. The first experiment showing changes in DNA methylation in human cancer was performed on samples of human colorectal cancer that, when compared with matched normal mucosa samples isolated from the same patients, showed widespread hypomethylation [156,157].

Loss of genome-wide methylation, especially in repetitive elements [158], promotes genome instability, considered as a major hallmark of cancer [159,160]. Genome instability contributes to the genetic diversity observed in most solid cancers [30].

After detection of global DNA hypomethylation in cancers, the epigenetics of human cancer has received increasingly attention with a growing knowledge of a variety of specific epigenetic aspects, including hypomethylation, hypermethylation, loss of imprinting and chromatin modification **(** [161,157,162, 163,164]. In particular, also hypermethylation in specific genes (promoters) was found to be related to cancer induction [165].

Methylation in the transcribed regions of genes is positively correlated with gene expression, and demethylation in these areas could result in the reduced expression of some genes [166]. On the

contrary, the gene hypermethylation, often involving normally unmethylated CpG[16] islands, can be associated with their transcriptional silencing and, if they are suppressor genes, their loss of function may be a key event contributing to the oncogenic process. [167,168,169]. For example, silencing of the BRCA1 gene by promoter hypermethylation occurs in primary breast and ovarian carcinomas, supporting a role for this tumour suppressor gene in sporadic breast and ovarian tumorigenesis [170]. A recent meta-analysis of the altered genes in colorectal cancer reinforces their involvement in tumorigenesis [256]. It has been evaluated that more than 300 genes and gene products are epigenetically altered in various human cancers [171].

Although the alteration of DNA methylation patterns in cancers has been recognized for several decades, the causal relevance of these changes to the cancer process has only later been accepted [172,173,174,175]. While research in cancer epigenetics was initially focussed on DNA methylation abnormalities, particularly on CpG island promoter methylation [257], other players have eventually emerged, a not unexpected result given that probably about 40% of human genes do not contain CpG islands in their promoters [176].

Besides aberrant DNA methylation, that is the most well studied epigenetic changes in cancer cells, it was found that histone modifications and chromatin remodelling are also implicated in cancer [177,178], so that the characterization of cancer epigenome should be based only on global changes in DNA methylation, but also on histone modification patterns as well as altered expression profiles of chromatin modifying enzymes [76]. Importantly, aberrant activity of histone-modifying factors may promote cancer development by misregulating chromatin structure and activity, an example of which is frequently found in human leukaemia [162].

In recent years, there has been a tremendous and growing interest to investigate the role of dysregulation of ncRNAs, notably miRNAs, in normal cellular functions as well as in disease processes. There is now emerging evidence that miRNAs and lncRNA are involved in the development and progression of leukaemia and cancer [179,180,181]. For example, transcriptional regulation of miRNAs has been found to control the pathogenesis of breast cancer via tumour suppressor targeting [182]. A puzzling aspect is that miRNA can act as either tumour suppressors or oncogenes [183,184,185,186].

Not surprisingly, epigenetic effects are also implicated in radiation-induced cancers. A variety of epigenetic changes have been observed in irradiated biological systems, given that the oxidative damage caused by ionising radiation, mentioned in a preceding Section, can influence DNA methylation in several ways, causing both global hypomethylation leading to chromosomal instability; and/or promoter hypermethylation leading to silencing of the tumour suppressors [86]. Several lines of evidence support this link. It has long been known that the epigenetic changes affecting a substantial fraction of irradiated cells can destabilize their genomes suggesting that the elevated postirradiation mutation rates in cell progeny, rather than gene-specific initial mutations, act to drive radiation tumorigenesis [187,188].

Hypermethylation of tumour suppressors genes have also demonstrated in murine models of radiation-induced lymphoma, in lung tumours of rats induced by exposure to Pu-239, and in human lung adenocarcinoma occurred in workers of the Russian MAYAK plutonium plant [189].

Alterations in miRNA expression may occur following exposure to several stress-inducing anticancer agents including ionizing radiation, etoposide, and hydrogen peroxide (H_2O_2). Dysregulation of a family of miRNA was found in Ptch1+/- mice that are highly susceptible to radiation-induced medulloblastoma [190]. The miRNA related epigenetic changes have been proposed to be the missing link between radiation exposure, radiation-induced genomic instability, and radiation-induced carcinogenesis [44].

These findings are consistent with the general notion that typically miRNAs involved in radiation tumorigenesis are deregulated, and this deregulation is believed to alter the expression of protein-coding

[16] A CpG island is a location within a DNA sequence (typically1000-kb stretch of DNA) that contains a high level of CpG sites, sometimes located consecutively. Transcriptionally active regions of the genome are usually CpG rich, and many regions of the genome contain large clusters of CpG dinucleotides. Methylation of CpG sites is a critical factor affecting gene transcription because of its ability to directly silence gene expression. CpG islands occupy about 60 % of human gene promoter regions.

mRNA, thereby favouring uncontrolled tumour cell growth, in some cases by decreasing tumour suppressor expression [191]. It should be noted that most of the investigations focussed to the relationship between radiation-induced cancer and miRNA changes have been obtained using rodent models, while relatively fewer studies have been performed on human cancers.

In rat mammary cells it was found that the frequency of initiation (the first step in oncogenesis) induced by 7 Gy was much higher than specific locus mutations, a finding supporting the hypothesis that radiogenic initiation of cancer in these cells is an epigenetic event [192,193]. Even though the doses used are much higher than those inducing the stochastic effects considered in radiation protection, the observation that radiation exposure, besides its mutational effects, may result in modifications of the epigenome has suggested that the onset of malignancies after irradiation is not caused by gene mutations only, but it also involves epigenetic changes.

DNA methylation was found in plants exposed in Chernobyl with a dose-dependent manner, suggesting a fundamental epigenetic role [194,195]. A study focussed on human lung adenocarcinoma in radiation-exposed workers compared to non-worker controls of the Russian MAYAK weapons-grade plutonium plant, showed that methylation at a particular gene (*CDKN2A*, coding for tumour suppressor proteins) occurred more often in workers than in non-worker controls, and the prevalence of this methylation exhibited a dose–response with radiation internal exposure dose [196]. Methylation of *CDKN2A*, involving silencing of this tumour suppressor gene, has also been linked to reactive oxygen species produced by radiation exposures [197].

Interestingly, upregulation of a specific miRNA was recently found in radiation-associated breast cancer tissue samples from Chernobyl radiation-exposed female clean-up workers [198].

9. Possible epigenetic role in radiation-induced cognitive effects

The possibility was recently considered that epigenetic mechanisms significantly contribute to the functional and structural neurological changes that lead to the adverse cognition effects of ionising radiation. Indeed, clinicians have known for decades that patients subjected to cranial radiotherapy for the control of brain malignancies develop severe and progressive cognitive deficits (see, e.g., [199]). Investigations showing cognitive/behavioural deficits caused by charged particles (relevant for space radiation) in rodent models have been carried out to understand the possible limitation to human exploration of our solar system (see the reviews in [200,201]). Interestingly, in rats a correlation was recently found between behavioural changes and epigenomic remodelling in the hippocampus [202] and between adverse effects on cognition of space relevant irradiation and epigenetic aberrations consisting in increased levels of the DNA methylating enzymes [203].

The notion that epigenetic mechanisms play a key role in cognitive responses to stress in animals, and in how they consolidate memories associated with a stressful event [204], poses the question whether there is a possible and interesting link with the mechanisms involved in the response to ionising radiation.

10. Some perspectives

10.1. Epigenetics and the low dose[17] issue

In spite of the great advances in our knowledge of biological effects of ionising radiation since their discovery, there remains considerable scientific uncertainty about the health risks of exposures to ionising radiation at low doses and/or low dose rates, typical of those encountered in the workplace, in the environment and in diagnostic medicine with potential impact in our every day life [28,29,205].

[17] The term ‘‘low dose’’ has many different interpretations in different contexts, but in terms of microdosimetry it is an absorbed dose such that a single cell or nucleus is very unlikely to be traversed by more than 1 track so that the number of affected cells is proportional to absorbed dose. However the definition of “unlikely” is subjective. A conservative definition, [206], corresponds to a mean number of 0.2 tracks per cell (or per cell nucleus) meaning that less than 2% of the cells will be subject to traversals by more than one radiation track. This would correspond to a dose of only 0.2 mGy of low-LET radiation [207].

Much of the damage from low dose radiation, like normal metabolism, is from Reactive Oxygen Species (ROS). Many data show that the biological responses to high and low doses of radiation are not only quantitatively, but also qualitatively, different. This holds in particular for gene expression profiles. Gene expression changes were established as an early indicator of cellular responses to low-dose radiation in a human myeloid tumour cell line [208]. Subsequently many other data were accumulated for a variety of biological systems, including human models [258] and human tissue irradiated *in vivo*, where however a considerable individual variability in radiation response was observed [209].

Considering the role of epigenetics in gene expression, it is not surprising that evidence is also growing for a relevant role of epigenetics in low dose response. In recent years, several lines of evidence have been accumulating that low dose effects of ionizing radiation involve formerly unexpected cellular phenomena such as non-targeted and delayed radiation effects. These effects clearly contradict the classical paradigm of radiation biology assuming that all radiation effects on cells, tissues and organisms are due to the direct action of radiation on DNA [27].

Even if a few reports suggest that genomic instability can be induced by low doses of low-LET radiation; more consolidated data suggest that instability is not induced by doses of less than 0.1–0.2 Gy, and in some cases higher doses, of low-LET irradiation either *in vivo* or *in vitro*, except in transformed or otherwise abnormal cells [110]. Reviews of the experimental literature indicate a likely threshold for the induction of transmissible instability of 0.5 Gy low-LET radiation while, generally, high-LET radiation is considered to be more effective [110].

"Beneficial" effects of low dose irradiation, associated to DNA hypermethylation, have been observed in the offspring of mice when they are irradiated with low doses during early gestation [210], an effect that can be classified as "hormesis" rather then as adaptive response. Changes in cell metabolism and radiosensitivity have been found in cells grown at very reduced radiation background suggesting that very low level of chronic exposure, such as the natural background, may trigger defence mechanisms without genetic change, therefore by epigenetic mechanisms [211] (see Section 10.6).

There have been accumulated many data on hormesis and adaptive response after low dose exposure both *in vitro* and *in vivo* (see, e.g., the review in [205]). Specific gene modulations were observed as a result of chronic low-LET irradiation of mice at low doses [212]. Low dose irradiation of a particular strain of mice during early gestation resulted in hypermethylation at a specific locus in the male offspring with beneficial health effects in terms of developing obesity, cancer, and diabetes in adulthood. [210]. A role of DNA hypermethylation was suggested to be involved in adaptive response induced by chronic low-dose γ-irradiation of human B lymphoblast cells. [213].

In conclusion, there are now many lines of evidence for a cellular response to low dose of radiation controlled in part by gene expression networks.

10.2. Development of epigenetic markers

Epigenetic modifications can be employed as disease biomarkers, since they provide information about gene function, thus explaining or describing differences among phenotypes. In addition, epigenetic biomarkers can incorporate information regarding the effects of the environment and lifestyle on health and disease, and monitor the effect of therapies [214].

Methylated DNA sequences, histone modification patterns and miRNA regulation are three types of potential biomarkers that can be used in principle at any stage of a disease and can be associated with its cause or latency (risk biomarkers), onset (diagnostic biomarkers), clinical course (prognostic biomarkers), or response to treatment (predictive biomarkers). A good review of ionizing radiation biomarkers for potential use in epidemiological studies, including epigenetic biomarkers, has been published in 2012 [215].

Epigenetic biomarkers are currently being used for early detection of cancer and research efforts are made to continue developing predictive biomarkers not only for cancer diagnosis but also for prediction of prognosis and monitoring of response to cancer therapy, including radiotherapy.

This is particular true for DNA methylation biomarkers as they proved useful for early cancer detection and may have great potential in personalized treatments [216] and for classifying cancer and predicting their clinical course because of their relation to tumour progression and metastasis [217]. The rationale is that early aberrant DNA methylation states occurring during transformation appear to be retained during tumour evolution and differences among different regions of a tumour reflect the history of cancer cells and their response to the tumour microenvironment [115]. Therefore, understanding the molecular events that initiate and maintain epigenetic gene silencing could lead to the development of clinical strategies for the prevention and therapy of cancer [218].

In effect, identification of epigenetic differences in fibroblasts that contribute to the onset of fibrosis after radiotherapy have suggested the use of epigenetic markers for implementing personalized radiotherapy and improving antifibrotic treatment [219] and aberrant DNA methylation of specific genes was used to characterize the uterine carcinosarcoma [220].

Even if several studies identified putative epigenetic cancer biomarkers, some of which have been commercialized (Costa-Pinheiro et al 2015) [221], still the clinical use of them is very limited because there are a number of limitations to overcome, such as the poor specificity and sensitivity and the need of standardized methodologies for DNA methylation profiling [216, 221].

Histone modification patterns could also provide prognostic and diagnostic information in cancer [222]. A fundamental issue here is to understand when and how these changes correlate with alteration in gene activity. The published data on the effects of irradiation on methylation, acetylation, ubiquitination and other post-translational modifications of histones almost exclusively refer to high doses using *in vitro* models and not all the players in these processes have yet been fully characterised so that further studies are needed to assess whether they can serve as biomarkers for the effects of low dose exposure in humans [215].

Discovery of miRNAs provided new potential clinical tools due to their ability to regulate relevant genes and signalling pathways. They may represent new molecular markers indicative of radiation exposure and of radiation response. It should be considered that microenvironment-mediated epigenetic perturbations play an important role in cancer development [49], so that alterations in the tissue microenvironment caused by ionising radiation can contribute to carcinogenesis and can also affect the tissue response to anticancer therapy [223]. MiRNAs changes within a tumour microenvironment are likely of great importance. In humans miRNAs are released into the circulation within microscopic vesicles (exosomes) and may mediate the cross-talk between cancer cells and the surrounding cells [224]. Advances in understanding the underlying biology of these interactions are critical to guiding rational approaches to designing novel treatment strategies.

In several cases miRNA expression profiling has been evaluated as a potential diagnostic biomarker [163,53,225] and as a prognostic biomarker of cancer [226,147, 179,227,228]. MiRNAs could also represent a novel therapeutic target in cancer therapy [147, 227]. For example, it was found that specific classes of miRNAs modulate the radiosensitivity of cancer cells [229,230].

An important issue in this kind of research is the need to understand the biological and functional meaning of the changes observed in the miRNA expression patterns in response to ionizing radiation, and this requires the knowledge of the functions (including their targets) of an increasing number of mammalian miRNAs. A promising line of investigation is the analysis of miRNA responses in selected biological systems that have manifested cancers after exposure to ionising radiation. In this way it could be possible to get new information about the mechanisms of radiation-induced cancer, with a great impact on radiation protection and on radiation biology in general.

It was found that miRNA expression signatures in mouse peripheral blood induced by irradiation with protons, γ-rays, or ^{56}Fe-ions are radiation type- and dose-specific suggesting that blood miRNAs could be used as *in vivo* biomarkers that can distinguish between low- and high-LET radiation, as well as between different doses of ionising radiation. [231,232].

Epigenetic changes have been considered as biomarkers of individual radiosensitivity and risk for radiation protection purposes. Because multiple end points and tissues are involved in the response to radiation, a multi-marker approach is believed to be necessary to provide this kind of information [215].

Epigenetic biomarkers can also be employed for environmental monitoring and radiotoxicology of internal emitters. Published data suggest they could be very promising to assess metabolomic signatures identifying contaminated from uncontaminated individuals [233]. "Epigenetic memory" has been proposed to be utilised by regulators in retrospective monitoring of the environment and to monitor a person's exposure history [234].

Epigenetic biomarkers could in principle be used by NASA for monitoring the biological impact of cumulative high-LET radiation exposure and the associated health risks encountered by astronauts in deep space [66]. They could also provide a long-term memory of prior space radiation exposure [65].

10.3. Epigenetics in radiation risk assessment: cancer induction

Cancer, initially recognized as a genetic disease, is now known to involve epigenetic abnormalities along with genetic alterations. Epigenetic changes have become increasingly recognized as important factors contributing to cancer development, so that understanding how ionising radiation affects the human epigenome could be a critical component for a complete understanding of the mechanisms of radiation-induced cancer. Therefore, models of the mechanisms involved in carcinogenesis should ultimately incorporate both elements when estimating radiation risk. [30]. In general it has become clear that gene function and cellular processes can be regulated at the epigenetic level and the extent to which radiation affects epigenetic states that relate to carcinogenesis needs to be elucidated [235].

Epigenetic considerations also impact on individual susceptibility to radiation-induced cancer. Assessment of individual variability in cancer risk is a key area to address for radiation protection. It is recognized that differences in radiation sensitivity between individuals, or groups, may relate to gender, age at exposure, state of health, genetic and epigenetic make-up, lifestyle, and age attained and that such differences, if significant, raise the ethical and policy question as to whether some individuals or groups are inadequately protected by the present system and regulations [235]. Therefore, research is required to settle this issue, and the role of epigenetic traits should be clarified.

10.4. Epigenetics in radiation risk assessment: transgenerational effects

Many examples have been reported for trans-generational epigenetic effects in which radiation exposures lead to heritable phenotypic changes that pass through male, female and sometimes both germ-lines [236].

Immediately relevant questions are whether the effects are common or rare, and whether they are long-lasting or transient. Research for answering these questions must face the difficulty of discerning epigenetic inheritance from conventional genetic effects.

Do transgenerational effects impact human phenotypic variation and disease risk? The answers to these questions will not only reveal insights about the mechanisms of transgenerational epigenetic effects, but will also provide evidence for the relative importance of this exceptional and largely unexplored mode of inheritance.

A relatively new problem is represented by treatment-related complications in radiation therapy. Although modern cancer radiotherapy has led to increased patient survival rates, radiation, among various complications, also poses a threat to the progeny of exposed parents, due to possible transgenerational genome instability that is linked to transgenerational carcinogenesis. The observed changes in mice were indicative of a profound epigenetic dysregulation in the offspring [131].

To determine the possible contribution to long-term or transgenerational effects of mechanisms governing the activation or the repression of the epigenome of organisms exposed chronically to low-dose ionising radiation even further experiments are planned in the framework of the COMET Project

[140]. Two biological models have been chosen to conduct these experiments: one vertebrate model (zebrafish, *D. rerio*) and one plant model (*Arabidopsis thaliana*) also used for field studies in Chernobyl and in Fukushima areas.

If transgenerational effects of radiation would be demonstrated to apply to humans, it may have implications in radiation protection when estimating the hereditary risks (i.e., the risk of induction of genetic disease expressed in future generations) of ionising radiation in humans populations. According to the current risk assessment system they are quantified as the harmful genetic effects to the descendants of those exposed resulting from the induction of germline mutations and their transmission over generations. [18]. This implies that mutation induction in directly exposed cells is regarded as the cause of this risk for humans. Indeed, since epidemiological studies have not provided clear evidence of heritable effects of radiation exposure in humans, current genetic risk estimates for radiation [145] are derived from measured germline mutation frequencies in mice[18]. If also the results of animal and cellular studies on epigenetic transgenerational destabilization of the genome would apply to human populations, then the hereditary risk could be greater than predicted previously.

Direct examination of the human data could settle the problem but, as mentioned in a previous section, they are very scarce and controversial. An extensive review published few years ago concluded that human health has not been significantly affected by transgenerational effects of radiation and that these effects, if any, are possibly restricted to relatively short times post-exposure, when in humans conception is likely to be rare [127].

However, further research is needed to resolve the discrepancies between human and animal/cellular data. As new data are being produced, it is necessary to continue their review so as to reach a consistent picture of this kind of effects. Implications of this research in radiation protection are different from that in radiation biology. Concerning the first aspect, it must be considered that in the current risk assessment hereditary risks are only a minor contribution (about 4%) of the total detriment due to stochastic effects caused by ionising radiation exposure, the major contribution being represented by cancer induction in the irradiated person, i.e., by somatic effects [18]. Therefore, a possible further contribution of transgenerational genome instability to the increase in mutation rates in the offspring of irradiated parents is likely not to affect much the present radiation protection practice while it could be of great interest for improving our knowledge in radiation biology.

10.5. Epigenetics in cancer radiotherapy

The initiation and progression of cancer is controlled by both genetic and epigenetic events. Unlike genetic alterations, which are almost impossible to reverse, epigenetic aberrations are potentially reversible, allowing the malignant cell population to revert to a more normal state.

Besides their application as biomarkers during the cancer treatment, epigenetic alteration can also be candidate targets for chemotherapy. In effect, with the advances in knowledge on the mechanisms and targets involved in the epigenetic regulation of gene expression, the utilization of “epi-drugs” that target specific enzymes has emerged as a possible approach to cancer therapy [149,237].

Applications of epigenetics in radiotherapy have not been studied extensively, although epigenetic alterations are interesting candidates as radiosensitisers. Epigenetic radiosensitization may be achieved by the regulation of chromatin structure modifications, or by epigenetic manipulation of genes involved in cell cycle, apoptosis or DNA repair [238]. Many epigenetic drugs have been considered and studied as radiosensitisers with varying results. (see [239] for a review).

It has to be considered that radiotherapy itself introduces epigenetic alterations so that it can be hypothesized that they influence the treatment outcome [240]. Given that tumour relapse can be attributed to a population of cancer stem cells (CSC) that survives radiotherapy, analysis of this cell population might suggest tactics to personalize treatment. For example it was shown that irradiating

[18] It was argued that “Experimental studies in plants and animals have demonstrated that radiation can induce hereditary effects, and humans are unlikely to be an exception in this regard” [25].

prostate cancer cells induces stable epigenetic changes stimulating a gene transcription in CSC through its methylation. Inhibiting this event triggered apoptosis and promoted radiosensitization, showing that epigenetic therapies may restore the cytotoxic effects of irradiation in radioresistant CSC populations [241].

The interaction between radiotherapy and epigenetic changes should be extensively studied, with the definition of the specific epigenetic alterations induced by radiation at the molecular level in various cancer types [81]. Such information is essential for transforming the promising results into clinical practice allowing tailor-made treatment on an individual basis.

10.6. Epigenetics, background radiation and evolution

Life has evolved on Earth for about 4 billion years in the presence of the natural background of ionising radiation even if it was not always the same as today. Without it, life on Earth could not have existed or would not exist in the present form. The Earth's crust itself contains radionuclides and the Earth is also bombarded by high-energy particles originating in outer space (cosmic radiation). Life is shielded against cosmic particles by the Earth's magnetic field and by the atmosphere layer but some radiation reaches the biosphere as a consequence of the primary particle interactions that generate secondary particles in the atmosphere[19].

Today the annual dose due to natural background on average approaches 1 mSv, with cosmic contributions slightly less than the terrestrial one [242]. For human organisms there is an internal exposure due to inhalation (mainly radon) and ingestion of naturally-occurring radionuclides (K-40 and others) that adds to the mentioned external exposure so that the total average annual dose is evaluated to be 2.4 mSv [242].

In the late 20s, it was suggested that variations in cosmic radiation, in addition to possible contribution to organic evolution, could have affected the evolution of life on earth (see the review [243]). It should be considered that probably in the past the cosmic rays on Earth have experienced many fluctuations due to explosions of supernovae in the nearby interstellar space and to variations in solar wind.

Despite the fact that the natural radiation background is extremely small, nevertheless it may be significant enough for living organisms to sense it and respond to it, keeping memory of this exposure. Controlled long-term experiments with model organisms, conducted in underground laboratories where conditions with no or largely reduced background radiation are realized, compared with normal conditions at natural radiation background, can provide basic information for understanding the evolutionary responses of living organisms to this background (see the review [244]).

The pioneering works of Planel et al. [246,247] have shown that the growth of protozoan and cyanobacterium cells was inhibited when they were cultured in a low-radiation environmental laboratory in the Pyrenees Mountains. Mammalian and bacterial cells grown under reduced radiation environmental conditions at the Waste Isolation Pilot Plant in New Mexico have shown upregulation of stress-related genes, including those for catalase production [248,249]. A series of experiments in the Gran Sasso underground Laboratory of the Italian National Institute of Nuclear Physics using various *in vitro* cultured cell lines have shown that cultures kept in this strongly reduced background, when compared with similar cultures in normal background, are less efficient in ROS scavenging, are down-regulated in genes involved in protection from oxidative damage, and are more susceptible to subsequent radiation-induced damage [250,251,252,211]. Some of these experiments are conducted in such a way to minimize the influence of genetic effects, such as induction and selection of radiosensitive mutants. Recent experiments with a more complex organism, the fruit fly, indicated that reduction of radiation background significantly increased the fly lifespan, but at the same time it reduced both male and female fertility [253].

[19] Studying the effects of cosmic radiation on human organisms, even outside the protection offered by the earth's atmosphere and magnetic field, has presently a great importance for assessing the radiation risks during human space travel [245] .

Overall, these results are consistent with the hypothesis that the natural radiation background is capable to stimulate defence mechanisms that are acquired by epigenetic regulation, an explanation that finds support in the already explored epigenetic origins of low-dose radiation responses such as the adaptive response and other non-targeted effects.

This kind of investigations is relevant for at least two aspects: a) for a better knowledge of the complex response of living organisms to low dose/low dose rate radiation exposure, for which a plethora of data are accumulating showing non-linear effects and departure from the linear, no-threshold (LNT) relationship assumed in radiation protection; b) for improving our knowledge about the role played by the natural radiation in life evolution, and about the possible role of radiation-induced epigenetic mechanisms in heritable adaptive gene expression.

Conclusions

The emerging picture of the cell response to ionising radiation speaks in favour of a complex response to a variety of radiation-induced signals with perturbations at the cellular and supracellular levels. It must not be surprising that no general agreement has yet been reached about the overall biological effect of ionising radiation, especially at low doses where beneficial and detrimental effects mix together making difficult any prediction of the health impact at the human organism level. The representation of ionising radiation as a double-edged sword, that is frequently used to visualize the fact that every radiotherapy has side effects because of normal tissue complications, may equally apply to every situation. The biological effects of ionising radiation on cells, frequently assumed to be a “radiation damage” by definition, can induce various cell responses, including triggering of defence mechanisms. A central problem is understanding how the genetic and epigenetic factors modulate this radiation response and which their respective roles are.

The revolution in biotechnology that has occurred over the last decade has provided researchers with access to a wealth of new methods and tools to investigate epigenetic effects. Several methods are now available to study methylation at the CpG sites which are found in the proximal promoter regions in the mammalian genome [254]. These tools will help in settling several outstanding questions, such as whether radiation exposure causes DNA methylation changes at specific regions of the human genome, and whether there are regions that are more prone to radiation-induced methylation “damage”. In addition, the widespread availability of methods for analysis of miRNAs and histones makes them also be a focus of study besides DNA methylation, and perhaps they will prove to be equally important mechanisms as, or even more important than, DNA methylation [255].

In conclusion, epigenetics opens a new avenue to answer important basic science questions. During the past years numerous scientific articles, meetings and new journals have been devoted to this subject, encompassing some of the most exciting contemporary scientific questions. Its popularity is continuously increasing, probably also because it is seen as an antidote to the idea that we are hard-wired by our genes. As such, epigenetics is considered as a revolution in evolutionary biology. At the same time, since the study of major human diseases can benefit from knowledge and control of epigenetic mechanisms, it is clear that epigenetics is also rapidly moving to the forefront of biomedical research.

Ionising radiation has clearly been demonstrated to be an agent capable to induce, besides genetic, also epigenetic effects, whose knowledge appears essential for understanding many important features of the mechanisms of radiation action on living systems, with important implications in radiation science and technology, and also in other scientific fields, including evolutionary biology.

REFERENCES

1. J. F. Ward (1985), *Biochemistry of DNA Lesions.* Radiation Research: **104(**2s): 103-S111.
2. J.F. Ward (1994), *The complexity of DNA damage: relevance to biological consequences,* Int. J. Radiat. Biol. **66**: 427–432.
3. D.T. Goodhead , J.Thacker, R. Cox. (1993). *Weiss Lecture. Effects of radiations of different qualities on cells: molecular mechanisms of damage and repair*. Int J Radiat Biol.;**63**(5):543-56.
4. D.T Goodhead (1994), *Initial events in the cellular effects of ionising radiation: clustered damage in DNA.* Int. J. Radiat. Biol. **65**:7-17.
5. H. Nikjoo, P. O'Neill, W.E. Wilson, D.T Goodhead (2001), *Computational Approach for Determining the Spectrum of DNA Damage Induced by Ionizing Radiation*. Radiat. Res. **156**: 577–583.
6. K. M Prise, M Pinto, H. C Newman, B. D. Michael, (2001). *A review of studies of ionizing radiation-induced double-strand break clustering*. Radiat. Res. **156**: 572–576.
7. P. O'Neill, P. Wardman. (2009). *Radiation chemistry comes before radiation biology,* International Journal of Radiation Biology 85(1):9-25.
8. R.M. Anderson D.L. Stevens, D.T. Goodhead (2002), *M-FISH analysis shows that complex chromosome aberrations induced by α-particle tracks are cumulative products of localized rearrangements.* PNAS **99** (19):12167–12172
9. R.M. Anderson, S.J. Marsden, S.J. Paice, A.E. Bristow, M.A. Kadhim, C.S. Griffin, D.T. Goodhead (2003), *Transmissible and Nontransmissible Complex Chromosome Aberrations Characterized by Three-Color and mFISH Define a Biomarker of Exposure to High-LET α Particles*. Radiation Research **159**(1):40-48.
10. J.R. Savage and P.J. Simpson (1994), *FISH "painting" patterns resulting from complex exchanges*. Mutat. Res. **312**, 51–60
11. S. Ritter M. Durante (2010), *Heavy-ion induced chromosomal aberrations: a review.* Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 701 (1), 38–46.
12. B.D. Loucas, M. Durante, S. Bailey, M.N. Cornforth (2013), *Chromosome damage in human cells by γ-rays, α-particles and heavy ions: track interactions in basic dose–response relationships.* Radiat. Res., **179**: 9–20
13. B. Wallace (1958), *The Average Effect of Radiation-Induced Mutations on Viability in Drosophila melanogaster*. Evolution **12**(4):532-556
14. H. Nikjoo, P. O'Neill, W.E. Wilson, D.T. Goodhead. (2001), *Computational approach for determining the spectrum of DNA damage induced by ionizing radiation.* Radiat Res. Nov;**156**:577-83.
15. J. H Muller (1962), *Studies in Genetics,* Indiana University Press, Bloomington, Indiana.
16. I. Asimov (1964). *Biographical Encyclopedia of Science and Technology*, Doubleday, Garden City, New York, p. 525
17. D. T. Goodhead (2010), *New radiobiological, radiation risk and radiation protection paradigms.* Mutation Research, **687**(1–2):13–16.
18. ICRP (2007). *The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103*. Ann. ICRP 37 (2-4) Elsevier Science Ltd.
19. H. Nagasawa, J.B. Little (1992), *Induction of sister chromatid exchanges by extremely low doses of α-particles.* Cancer research, **52**(22):6394-6.
20. A.R. Snyder (2004), *Review of radiation-induced bystander effects*. Human and Experimental Toxicology, **23**(2): 87-89.
21. W.F. Morgan, (2003a), *Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation-induced genomic instability and bystander effects in vitro*, Radiat. Res. **159**: 567–580.
22. W.F. Morgan (2003b), *Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects*. Radiat Res **159**:581–596.
23. M. Kadim, S. Salomaa, E. Wright, G. Hildebrandt, O.V. Belyakov, K.M. Prise,M.P. Little (2013), *Non-targeted effects of ionising radiation-implications for low dose risk*. Mutat. Res. **752**, 84–98.

24. G. Olivieri, J. Bodycote, S. Wolff (1984), *Adaptive response of human lymphocytes to low concentrations of radioactive thymidine.* Science **223**:594–597.
25. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), UNSCEAR 2006 Report, vol. II, Annex C, *Non-Targeted and Delayed Effects of Exposure to Ionizing Radiation*, New York, United Nations,2006, pp. 1–79
26. B.J. Blyth, P.J. Sykes, Radiation-induced bystander effects: what are they, and how relevant are they to human radiation exposures?, Radiat Res. 176 (2011) 139–157.
27. D. Averbeck (2010), *Non-targeted effects as a paradigm breaking evidence.* Mutation Research **687**(1–2),7–12.
28. HLEG. *High Level and Expert Group Report on European Low Dose Risk Research-Radiation Protection*. European Commission EUR 23884, Office for Official Publications of the European Communities (2009)
29. M. Belli, M. A. Tabocchini, J-R. Jourdain, S. Salomaa, J. Repussard (2015), *The European initiative on low-dose risk research: from the HLEG to MELODI*. Radiation Protection Dosimetry **166**(1-4):178-181.
30. M.H. Barcellos-Hoff and A. L Brooks (2001), *Extracellular Signaling through the Microenvironment: A Hypothesis Relating Carcinogenesis, Bystander Effects, and Genomic Instability*.Radiat. Res. **156**:618–627.
31. B. Djordjevic (2000), *Bystander effects: a concept in need of clarification*. Bioessays **22**(3): 286-290)
32. Campa, M. Balduzzi , V. Dini, G. Esposito, M.A. Tabocchini (2015), The complex interactions between radiation induced non-targeted effects and cancer. Cancer Letters 356(1): 126–136, doi.org/10.1016/j.canlet.2013.09.030.
33. M. Belli, O. Sapora, M.A. Tabocchini (2002), *Molecular Targets in Cellular Response to Ionizing Radiation and Implications in Space Radiation Protection*. J. Radiat. Res., **43**:S13–S19.
34. J. W. Harper and S. J. Elledge (2007), The *DNA Damage Response: Ten Years Afte*r. Mol Cell **28**, 739-745.
35. A. Ciccia, S.J. Elledge (2010), *The DNA damage response: making it safe to play with knives*. Mol Cell **40**,179–204. doi:10.1016
36. Y. Yao and W. Dai (2014) Genomic Instability and Cancer. Carcinog & Mutagen. 5: 165-178. doi:10.4172/2157-2518.1000165
37. R.Freil and M.R. Fraga (2012), *Epigenetics and the environment: emerging patterns and implications.* Nature Reviews Genetics **13**, 97-109.
38. B. C. Christensen and C. J. Marsit, (2011), *Epigenomics in environmental health,* Front. Genet, **2**,1-10, https://doi.org/10.3389/fgene.2011.00084.
39. F. Pacchierotti and M. Spanò. (2015), *Environmental Impact on DNA Methylation in the Germline: State of the Art and Gaps of Knowledge*, BioMed Research International. **2015**, 1-23, http://dx.doi.org/10.1155/2015/123484.
40. J.A. Head, D.C. Dolinoy, N. Basu. (2012) *Epigenetics for Ecotoxicologists*. Environmental Toxicology and Chemistry, **31**(2), 221-227.
41. J.A., Law and S.E. Jacobsen (2010), *Establishing, maintaining and modifying DNA methylation patterns in plants and animals.* Nature Reviews. Genetics, **11**(3), 204–220. http://doi.org/10.1038/nrg2719.
42. W. Goetz, M.N. Morgan and J.E. Baulch (2011), *The effect of radiation quality on genomic DNA methylation profiles in irradiated human cell lines*. Radiat. Res. **175**(5): 575- 587.
43. S. Kaup, V. Grandjean, R. Mukherjee, A, Kapoor, E. Keyes, C.B.Seymour, C.E.Mothersill, P.N.Schofield (2006), *Radiation-induced genomic instability is associated with DNA methylation changes in cultured human keratinocytes*. Mutat Res **597**, 87-97.
44. U. Aypar, W.F. Morgan, J.E. Baulch (2011)), *Radiation-induced epigenetic alterations after low and high LET irradiations.* Mutation Research **707**:24–33.
45. I. Pogribny, J. Raiche, M. Slovack, O. Kovalchuk (2004), *Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes.* Biochem. Biophys. Res. Commun., **320**, 1253–1261.

46. J. Raiche, R. Rodriguez-Juarez, I. Pogribny, O. Kovalchuk (2004), *Sex- and tissue-specific expression of maintenance and de novo DNA methyltransferases upon low dose X-irradiation in mice.* Biochem. Biophys. Res. Commun., **325**, 39–47.
47. R. Tawa, Y. Kimura, J. Komura, Y. Miyamura, A. Kurishita, M.S. Sasaki, H. Sakurai, T. Ono (1998), *Effects of X-ray irradiation on genomic DNA methylation levels in mouse tissues*. J. Radiat. Res. **39**, 271–278.
48. R. Margueron, P. Trojer, D. Reinberg (2005), *The key to development: interpreting the histone code?* Curr. Opin. Genet. Dev. **15**, 163–176.
49. S. L. Berger, T. Kouzarides, R. Shiekhattar, A. Shilatifard (2009), An operational definition of epigenetics. Genes & Development **23**:781–783.
50. L .Mendez-Acuna, M.V. Di Tomaso, F. Palitti, W. Martinez-Lopez (2010), *Histone posttranslational modifications in DNA damage response.* Cytogenet Genome Res **128**:28–36.
51. N. B. Averbeck and M. Durante. (2011). *Protein acetylation within the cellular response to radiation.* Journal of Cellular Physiology 226:4, 962-967.
52. R.C. Friedman, K. K.-H. Farh, C.B. Burge, D.P. Bartel (2009), *Most mammalian mRNAs are conserved targets of microRNAs.* Genome Res. **19**(1), 92–105.. doi: 10.1101/gr.082701.108.
53. M Esteller . (2011) *Non-coding RNAs in human disease*. Nat Rev Genet.**12**(12): 861-74. doi: 10.1038/nrg3074.
54. A. Kraemer, N. Anastasova, M. Angermeier, K. Winkler, M. J. Atkinson, S. Moertl (2011). *MicroRNA-Mediated Processes are Essential for the Cellular Radiation Response*. Radiation Research, **176**,575-586.
55. A. Fatica, I. Bozzoni (2014), *Long non-coding RNAs: new players in cell differentiation and development.* Nat Rev Genet.**15**(1):7–21, doi: 10.1038/nrg3606.
56. J.L. Rinn, H.Y. Chang (2012), *Genome regulation by long noncoding RNAs*. Annu Rev Biochem. **81**,145–66. doi: 10.1146/annurev-biochem-051410-092902.
57. E. Anastasiadou, L.S. Jacob, F. J. Slack (2018), *Non-coding RNA networks in cancer*. Nature Reviews Cancer **18**, 5–18.
58. C. Metheetrairut and F. J. Slack (2013), *MicroRNAs in the Ionizing Radiation Response and in Radiotherapy*. Curr Opin Genet Dev. February **23**(1): 12–19.
59. M.A. Chaudhry, B. Kreger, R.A. Omaruddin (2010), *Transcriptional modulation of micro-RNA in human cells differing in radiosensitivity*. Int. J Radiat Biol. **86** (7): 569-583
60. K.M. Antwih, W.D. Gabbara, D.M. Lancaster, S.P. Ruden, S.P. Zielske. (2013) *Radiation-induced epigenetic DNA methylation modification of radiation-response pathways.* Epigenetics. **8**(8):839-48. doi: 10.4161/epi.25498.
61. I. Pogribny, I. Koturbash, V. Tryndyak, D. Hudson, S.M.L. Stevenson, O. Sedelnikova, W. Bonner and O. Kovalchuk (2005), *Fractionated Low-Dose Radiation Exposure Leads to Accumulation of DNA Damage and Profound Alterations in DNA and Histone Methylation in the Murine Thymus.* Mol Cancer Res **3**(10), 553-561; doi: 10.1158/1541-7786.MCR-05-0074
62. F. Lima, D. Ding, W. Goetz, A.J. Yang, J. Baulch (2014), *High LET 56Fe ion irradiation induces tissue-specific changes in DNA methylation in the mouse.* Environ. Mol. Mutagen. **55**, 266–277.
63. E. Nzabarushimana, I.R. Miousse, L. Shao, J. Chang, A.R. Allen, J. Turner, B. Stewart, J. Raber, I. Koturbash (2014), *Long-term epigenetic effects of exposure to low doses of 56Fe in the mouse lung.* J. Radiat. Res. (Tokyo) rru010.
64. I.R. Miousse, L. Shao, J. Chang, W. Feng, Y. Wang, A.R. Allen, J. Turner, B. Stewart, J. Raber, D. Zhou, I. Koturbash (2014), *Exposure to Low-Dose Fe-Ion Radiation Induces Long-Term Epigenetic Alterations in Mouse Bone Marrow Hematopoietic Progenitor and Stem Cells.* Radiation Research **182**, 92-101, https://doi.org/10.1667/RR13580.1
65. E.M. Kennedy, K.N. Conneely, P.M. Vertino (2014), *Epigenetic memory of space radiation exposure.* https://three-jsc.nasa.gov/articles/Vertino.pdf. Date posted: 07-30-2014. 5)
66. F.A. Cucinotta (2014), *Space Radiation Risks for Astronauts on Multiple International Space Station Missions.* PLoS ONE **9**, e96099.
67. M. Durante, F.A. Cucinotta (2008), *Heavy ion carcinogenesis and human space exploration.* Nat. Rev. Cancer **8**, 465–472.

68. R.C. Lee, R..L Feinbaum, V. Ambros (1993), *The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14*. Cell, **75**,. 843-854
69. R. W.Carthew and E.J.Sontheime (2009), *Origins and Mechanisms of miRNAs and siRNAs*. Cell **136**(4) 642-655.
70. A. Morano, T. Angrisano, G. Russo, R. Landi, A. Pezone, S. Bartollino, C. Zuchegna, F. Babbio, I. M. Bonapace, B. Allen, M. T. Muller, L. Chiariotti, M. E. Gottesman, A. Porcellini, . E. V. Avvedimento (2013), *Targeted DNA methylation by homology-directed repair in mammalian cells. Transcription reshapes methylation on the repaired gene.* Nucleic Acids Research, **42**, 804–821.
71. T. Templin, S.A. Amundson, D.J. Brenner, L.B. Smilenov (2011), *Whole mouse blood microRNA as biomarkers for exposure to γ-rays and ^{56}Fe ions.* Int. J. Radiat. Biol., **87**, 653–662.
72. T. Templin, E.F. Young, L.B. Smilenov (2012), *Proton radiation-induced miRNA signatures in mouse blood: Characterization and comparison with ^{56}Fe-ion and gamma radiation*. Int J Radiat Biol. **88**(7): 531–539.
73. S. Cerda and S.A. Weitzman (1997), *Influence of oxygen radical injury on DNA methylation* Mutation Research **386**(2), 141-152.
74. R. Franco, O. Schoneveld, A.G. Georgakilas, M.I. Panayiotidis (2008), *Oxidative stress, DNA methylation and carcinogenesis* Cancer Letters **266** (1):6–11
75. A.J.Bernal, D.C, Dolinoy, D. Huang, D.A Skaar, C. Weinhouse, R.L. Jirtle (2013). *Adaptive radiation-induced epigenetic alterations mitigated by antioxidants*. FASEB Journal, **27**(2), 665-671. doi: 10.1096/fj.12-220350
76. K.V. Donkena, C.Y.F. Young, D.J. Tindall (2010), Oxidative Stress and DNA Methylation in Prostate Cancer. Obstetrics and Gynecology International, **2010**, 1 14, doi:10.1155/2010/302051
77. D. Ziech, R, Franco,, A. Pappa, M.I.Panayiotidis (2011), *Reactive Oxygen Species (ROS)-Induced genetic and epigenetic alterations in human carcinogenesis*. Mutation Research, **711**(1–2), 167-173.
78. M. Dattilo, G. D'Amato, E. Caroppo, Y. Ménézo (2016), *Improvement of gamete quality by stimulating and feeding the endogenous antioxidant system: mechanisms, clinical results, insights on gene-environment interactions and the role of diet*. J Assist Reprod Genet, **33**:1633–1648.
79. Q. Wu and X. Ni (2015), *ROS-Mediated DNA Methylation Pattern Alterations in Carcinogenesis.* Current Drug Targets, **16**, 13-19.
80. I.Szumiel (2015), *Ionizing radiation-induced oxidative stress, epigenetic changes and genomic instability: the pivotal role of mitochondria*. .Int J Radiat Biol. **91**(1):1-12.
81. J.-G. Kim, M.-T. Park, K. Heo, K.-M. Yang, J.M. Yi (2013), *Epigenetics Meets Radiation Biology as a New Approach in Cancer Treatment.* Int. J. Mol. Sci. **14**, 15059-15073.doi:10.3390/ijms140715059
82. T. Jenuwein, C.D. Allis (2001), *Translating the histone code*. Science, **293**, 1074–1080.
83. Z. Herceg (2007), *Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors*. Mutagenesis, **22**(2), 91-103.
84. B. Lehnertz, Y. Ueda, A.A. Derijck, U. Braunschweig, L. Perez-Burgos, S. Kubicek, T. Chen, E. Li, T. Jenuwein, A.H. Peters (2003), *Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin*. Curr Biol. **13**(14),1192-1200.
85. M. Falk, E. Lukasova, S. Kozubek (2008), *Chromatin structure influences the sensitivity of DNA to gamma-radiation*, Biochim. Biophys. Acta **1783**, 2398–2414.
86. Y. Ilnytskyy, O. Kovalchuk (2011), *Non-targeted radiation effects-an epigenetic connection.* Mutat Res.;**714**,113–125.
87. X. Sun, Y. He, C. Huang, T.-T. Ma, J. Li (2013), *The epigenetic feedback loop between DNA methylation and microRNAs in fibrotic disease with an emphasis on DNA methyltransferases.* Cellular Signalling **25**, 1870–1876.
88. Y. Saito, G. Liang, G. Egger, J.M. Friedman, J.C. Chuang, G.A. Coetzee, P.A. Jones (2006), *Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells*. Cancer Cell **9,** 435–443.
89. J.C. Chuang, and P.A. Jones (2007), *Epigenetics and MicroRNAs Pediatric Research* **61**, 24R–29R.

90. A. Lal, Y. Pan, F. Navarro, D.M. Dykxhoorn, L. Moreau, E. Meire, Z. Bentwich, J. Lieberman, D. Chowdhury (2009), *MiR-24- mediated downregulation of H2AX suppresses DNA repair in terminally differentiated blood cells*. Nat Struct Mol Biol. **16**(5), 492-498.
91. A. Shkumatava, A. Stark, H. Sive, D.P. Bartel. (2009). *Coherent but overlapping expression of microRNAs and their targets during vertebrate development.* Genes & Development **23**:466–481.
92. R.C. Friedman, K.K. Farh, C.B. Burge, D.P. Bartel (2009), *Most mammalian mRNAs are conserved targets of microRNAs.* Genome Res., **19**, 92–105.
93. F. Fuks (2005), *DNA methylation and histone modifications: teaming up to silence genes*. Curr. Opin. Genet. Dev. **15**, 490-495.
94. S.A Lorimore, P.J Coates, E.G Wright (2003), *Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation*. Oncogene **22**(45):7058-7069.
95. L. Huang, P.M. Kim, J.A. Nickoloff, W.F. Morgan. (2007), *Targeted and nontargeted effects of low-dose ionizing radiation on delayed genomic instability in human cells.* Cancer Res. **67**(3), 1099-1104.
96. J.L. Schwartz (2007). Variability: the common factor linking low dose-induced genomic instability, adaptation and bystander effects. Mutat. Res. **616**, 196–200.
97. M. B. Sowa, W. Goetz, J.E. Baulch, A.J. Lewis, W. F. Morgan (2011), *No evidence for a low linear energy transfer adaptive response in irradiated RKO cells.* Radiation Protection Dosimetry, **143**, 311–314, doi:10.1093/rpd/ncq487.
98. W.F. Morgan, M.B. Sowa (2015), *Non-targeted effects induced by ionizing radiation: Mechanisms and potential impact on radiation induced health effects*. **356**, 17-21.
99. Campa, M. Balduzzi , V. Dini, G. Esposito,M.A. Tabocchini (2015), The complex interactions between radiation induced non-targeted effects and cancer. Cancer Letters 356(1): 126‑136. Published online: October 28, 2013.
100. I. Koturbash, A. Boyko, R. Rodriguez-Juarez, R.J. McDonald, V.P. Tryndyak, I. Kovalchuk, I.P. Pogribny, O. Kovalchuk (2007), *Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo.* Carcinogenesis **28**, 1831–1838.
101. M. Mancuso, E. Pasquali, S. Leonardi, M. Mancuso, E. Pasquali, S. Leonardi, M. Tanori, S. Rebessi, V. Di Majo, S.a Pazzaglia, M. P. Toni, M. Pimpinella, V. Covelli, A. Saran (2008), *Oncogenic bystander radiation effects in Patched heterozygous mouse cerebellum*. Proc Natl Acad Sci USA, **105**, 12445-12450.
102. O. Kovalchuk and J. E. Baulch (2008), *Epigenetic Changes and Nontargeted Radiation* Effects: Is There a Link? Environmental and Molecular Mutagenesis **49**, 16-25.
103. Y. Ilnytskyy, O. Kovalchuk (2011), *Non-targeted radiation effects-An epigenetic connection*. Mutation Research **714**, 113– 125.
104. U. Aypar, W.F. Morgan, J.E. Baulch (2011), *Radiation-induced genomic instability: are epigenetic mechanisms the missing link?* Int. J. Radiat. Biol. **87**(2),179-191.
105. M. Merrifield and O. Kovalchuk (2013). *Epigenetics in radiation biology: a new research frontier*. Front Genet.; **4**: 40. doi: 10.3389/fgene.2013.00040
106. L. Manti, M. Jamali , K.M. Prise, B.D. Michael, K.R. Trott (1997), *Genomic Instability in Chinese Hamster Cells After Exposure to X Rays or Alpha Particles of Different Mean Linear Energy Transfer*. Radiat Res **147** (1): 22-28.
107. J.B. Little. (1998), *Radiation-induced genomic instability*. Int J Radiat Biol.; **74** 663–671.
108. R.E. Rugo, J.T. Mutamba, K.. Mohan, T. Yee1, J. R. Chaillet, J.S. Greenberger, B. P. Engelward (2011), *Methyltransferases mediate cell memory of a genotoxic insult*. Oncogene 30(6): 751–756.
109. S. Kaup, V. Grandjean, R. Mukherjee, A, Kapoor, E. Keyes, C.B.Seymour, C.E.Mothersill, P.N.Schofield (2006), *Radiation-induced genomic instability is associated with DNA methylation changes in cultured human keratinocytes*. Mutat Res **597**, 87-97.
110. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2012), *Biological mechanisms of radiation actions at low doses.* New York, United Nations, 2012.
111. J.N. Filkowski, Y. Ilnytskyy, J. Tamminga, I. Koturbash, A. Golubov, T. Bagnyukova, I.P. Pogribny, O. Kovalchuk (2010), *Hypomethylation and genome instability in the germline of*

exposed parents and their progeny is associated with altered miRNA expression. Carcinogenesis. **1**(6):1110-5. doi: 10.1093/carcin/bgp300.
112. S. Tapio, V. Jacob (2007), *Radioadaptive response revisited*. Radiat Environ Biophys **46**, 1–12.
113. D. Wodarz, R. Sorace, N.L. Komarova (2014), *Dynamics of Cellular Responses to Radiation*. PLoS Comput Biol. **10**(4): e1003513.
114. G. Esposito, A. Campa, M. Pinto, G. Simone, M.A. Tabocchini, M. Belli (2010), *Adaptive response: modelling and experimental studies*. Radiation Protection Dosimetry, **143**(2-4), 320-324, doi:10.1093/rpd/ncq474.
115. M. Kim, J. Costello (2017), *DNA methylation: an epigenetic mark of cellular memory*. Experimental & Molecular Medicine (2017) **49**, e322; doi:10.1038/emm..10.
116. B. Zhu, D. Reinberg (2011), *Epigenetic inheritance: uncontested?* Cell Res. **21**, 435–441.
117. A. D'Urso and J.H. Brickner (2014), Mechanisms of epigenetic memory. Trends Genet. **30**(6), 230–236.
118. L.J. Gaydos, W. Wang, S. Strome (2014), *H3K27me and PRC2 transmit a memory of repression across generations and during development*, Science **345**(6203), 1515-1518.
119. E. Heard and R.A. Martienssen (2014), *Transgenerational Epigenetic Inheritance: myths and mechanisms*. Cell, 157(1), 95–109.
120. J.E. Baulch and O.G. Raabe (2005), *Gamma irradiation of Type B spermatogonia leads to heritable genomic instability in four generations of mice*. Mutagenesis, **16**, 17-23.
121. K.G. Luning, H. Frolen, A. Nilsson (1976), *Genetic effects of 239Pu salt injections in male mice.* Mutat. Res., **34**, 539–542.
122. T. Nomura. (1982), *Parental exposure to x rays and chemicals induces heritable tumours and anomalies in mice.* Nature **296** 575-577.
123. W.F. Morgan, (2003c), *Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure to ionizing radiation?* Oncogene **22**(45):7094-9.
124. A. Buisset-Goussen, B. Goussen, C. Della-Vedova, S.G. Christelle, A. Guillermin, C. Lecomte-Pradines (2014), *Effects of chronic gamma irradiation: a multigenerational study using* Caenorhabditis elegans. J Environ Radioact. **137**:190-197.
125. R.C. Barber, Y.E. Dubrova (2006), *The offspring of irradiated parents, are they stable ?* Mutat. Res. **598**, 50-60.
126. T. Nomura, L.S. Baleva, H. Ryo, S. Adachi, A.E. Sipyagina, N.M. Karakhan (2017), *Transgenerational effects of radiation on cancer and other disorders in mice and humans*. J Radiat Cancer Res, **8**:123-34.
127. M.P.Little, D.T.Goodhead, B.A. Bridges, S.D.Bouffler. (2013), *Evidence relevant to untargeted and transgenerational effects in the offspring of irradiated parents.* Mutation Research/Reviews in Mutation Research **753**(1): 50-67.
128. Y.E. Dubrova. (2003). *Radiation-induced transgenerational instability*. Oncogene **22**:7087–7093.
129. A. Soubry,C. Hoyo,R. L. Jirtle, S. K. Murphy (2014), *A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line*. Bioessays **36**: 359–371.
130. L. Paris, P. Giardullo, S. Leonardi, B. Tanno, R. Meschini, E. Cordelli, B. Benassi, M. G. Longobardi, A. Izzotti, A. Pulliero, M. Mancuso, F. Pacchierotti. (2015). Transgenerational inheritance of enhanced susceptibility to radiation-induced medulloblastoma in newborn Ptch1+/− mice after paternal irradiation. Oncotarget, 6(34), 36098-36112.
131. I. Koturbash, M. Baker, J. Loree, K. Kutanzi, D. Hudson, I. Pogribny, O. Sedelnikova, W. Bonner, O. Kovalchuk (2006), *Epigenetic Dysregulation Underlies Radiation-Induced Transgenerational Genome Instability In Vivo*. Int. J. Radiation Oncology Biol. Phys., **66**, 327–330.
132. R.L. Jirtle, M.K. Skinner. (2007). *Environmental epigenomics and disease susceptibility.* Nat Rev Genet **8**:253–262.
133. M. Merrifield and O. Kovalchuk (2013). *Epigenetics in radiation biology: a new research frontier*. Front Genet.; **4**: 40. doi: 10.3389/fgene.2013.00040
134. R. Barber, M.A. Plumb, E. Boulton, I. Roux, Y.E. Dubrova. (2002). *Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice*. Proc Natl Acad Sci USA **99**:6877–6882.

135. R.C. Barber, P. Hickenbotham, T. Hatch, D. Kelly, N.Topchiy, G.M. Almeida, G.D.D. Jones, G.E. Johnson, J.M. Parry, K. Rothkamm, Y.E. *Dubrova (2006),* Radiation-induced transgenerational alterations in genome stability and DNA damage. Oncogene **25**, 7336–7342.
136. H. Min , M. Sung , M. Son, I. Kawasaki, Y.-H. Shim (2017), Transgenerational effects of proton beam irradiation on Caenorhabditis elegans germline apoptosis. Biochemical and Biophysical Research Communications **490**(3), 608-615.
137. K.Gombeau, J-P Bourdineaud,. J-L. Ravanat,.V.Camilleri, I.Cavalie, O.Armant, V.Camilleri, I.Cavalie, M.Floriani, C.Adam-Guillermin (2017), *Epigenetic, histopathological and transcriptomic effects following exposure to depleted uranium in adult zebrafish and their progeny*. Aq. Toxicol. **184**, 14-25.
138. K.Gombeau, S.Pereira, J-L. Ravanat, V.Camilleri, I.Cavalie, J-P. Bourdineaud, C.Adam-Guillermin (2016), *Depleted uranium induces sex- and tissue-specific methylation patterns in adult zebrafish*. J. Env. Radioact., **154**, 25-33.
139. G. Elmhiri, C. Gloaguen, S. Grison, D. Kereselidze, C. Elie, K. Tack, M. Benderitter,P Lestaevel, A. Legendre, M. Souidi (2018), *DNA methylation and potential multigenerational epigenetic effects linked to uranium chronic low-dose exposure in gonads of males and females rats.* Toxicology Letters **282**. 64–70.
140. C. Adam-Guillermin, N.Horemans, D.Oughton, D.Spurgeon, K.Stark, S.Gashchak, V.Yoschenko, T.Hertel-Aas, S.Pereira, H. Vandenhove (2013), *COMET WP4 - Initial Research Activity State-of-the art on epigenetics and general approach. COMET Deliverable (D4.1),* Euratom 201.
141. C. Adam-Guillermin, K Gombeau, N.Horemans, D.Oughton, E. Lapied, D.Spurgeon, W. Tyler, K.Stark, S.Gashchak, V.Yoschenko, E.Saenen, S.Pereira, K.Nanba (2014), *Epigenetic changes induced in non-human organisms exposed to radionuclides : first results obtained in the WP4 COMET.* Workshop on Molecular Mechanisms of Radiation Toxicity at Chronic Low Dose Levels, St Catherine's College, University of Oxford, Oxford, UK, 10th – 12th December 2014.
142. S.N. Martos , W.Tang, Z. Wang (2015), Elusive inheritance: Transgenerational effects and epigenetic inheritance in human environmental disease. Prog Biophys Mol Biol. **118**(1-2):44–54.
143. O. Niwa (2003). *Induced genomic instability in irradiated germ cells and in the offspring; reconciling discrepancies among the human and animal studies*. Oncogene **22**:7078–7086.
144. A. Aghajanyan and I. Suskov (2009), *Transgenerational genomic instability in children of irradiated parents as a result of the Chernobyl Nuclear Accident*. Mutation Research, **671**, (1–2), 52-57.
145. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), UNSCEAR 2010 Report. New York, United Nations, 2011, pp. 1–14.
146. S.M. Gasser and E. Li, Eds. (2011). *Epigenetics and Disease*, Progress in Drug research **67**, Springer Basel AG 2011.
147. W. Timp, A. P. Feinberg (2013), *Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host*. Nature Reviews Cancer 13, 497–510
148. A.P. Feinberg, B. Vogelstein (1983), *Hypomethylation distinguishes genes of some human cancers from their normal counterparts.* Nature.; **301**:89–92.
149. L. Altucci, N. Clarke, A. Nebbioso, A. Scognamiglio, H. Gronemeyer (2005), *Acute myeloid leukemia: Therapeutic impact of epigenetic drugs.* International Journal of Biochemistry & Cell Biology **37**, 1752–1762.
150. P. C. Nowell (1976), *The clonal evolution of tumor cell populations*. Science **194**, 23–28.
151. P.A. Jones and M.L. Gonzalgo (1997), *Altered DNA methylation and genome instability: a new pathway to cancer?* Proc. Natl. Acad. Sci. USA **94**, 2103–2105.
152. C. Lengauer, K.W. Kinzler, B. Vogelstein (1997), *Genetic instability in colorectal cancers*. Nature **386**, 623–627.
153. J.-M. Zingg and P. A. Jones (1997), Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. Carcinogenesis, **18**(5), 869-882.
154. Y. Zhao and R. J. Epstein (2008), *Programmed genetic instability: a tumor-permissive mechanism for maintaining the evolvability of higher species through methylation-dependent mutation of DNA repair genes in the male germ line.* Molecular Biology and Evolution, 25(8), 1737– 1749.

155. R. Jaenisch, A. Bird (2003), *Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals*. Nat. Genet. **33,** 245–254.
156. A.P. Feinberg, B. Vogelstein (1983), *Hypomethylation distinguishes genes of some human cancers from their normal counterparts.* Nature.; **301**:89–92.
157. A.P. Feinberg, B. Tycko (2004), *The history of cancer epigenetics*. Nat Rev Cancer **4**: 143-153.
158. M.J. Hoffmann, W.A Schulz (2005), *Causes and consequences of DNA hypomethylation in human cancer.* Biochemistry and Cell Biology, **83**(3), 296-321
159. J. Rodriguez, J. Frigola, E. Vendrell, R.A. Risques, M.F. Fraga, C.Morales, V.Moreno ,M. Esteller, G. Capellà, M. Ribas, M.A. Peinado (2006), *Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers*. Cancer Res. **66**(17),8462-9468.
160. S. Negrini , V.G. Gorgoulis, T.D. Halazonetis (2010), *Genomic instability - an evolving hallmark of cancer.* Nature Reviews Molecular Cell Biology **11**, 220–228.
161. P. A. Jones, S. B. Baylin (2002), *The fundamental role of epigenetic events in cancer*. Nat. Rev. **3**, 415–428.
162. Z. Herceg (2007). *Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors.* Mutagenesis **22**, 91–103.
163. M. Esteller (2008), *Epigenetics in cancer*. NEJM, **358**:1148-1159.
164. E. McKenna (2013), *A Look at the Origins of Cancer Epigenetics*, Cancer Discovery, **3**:713.
165. S. B. Baylin , J. W. M. Hoeppener, A. de Bustros, P. H. Steenbergh, C. J. M. Lips, B. D. Nelkin (1986), *DNA Methylation Patterns of the Calcitonin Gene in Human Lung Cancers and Lymphomas*. Cancer Research **46**, 2917-2922.
166. R. Lister, M. Pelizzola, R.H. Dowen. R.D. Hawkins, G. Hon, J. Tonti-Filippini, J.R. Nery, L. Lee, Z. Ye, Q.-M. Ngo, L. Edsall, J. Antosiewicz-Bourget, Ron Stewart, Victor Ruotti, A.H. Millar, J.A. Thomson, B. Ren, J. R. Ecker (2009), *Human DNA methylomes at base resolution show widespread epigenomic differences. Nature*, **462**(7271), 315–322, doi:10.1038/nature08514
167. M.S. Mendonca, R.J. Antoniono, J.L. Redpath (*1993*), *Delayed Heritable Damage and Epigenetics in Radiation-Induced Neoplastic Transformation of Human Hybrid Cells*. Radiation Research **134**(2), 209-216.
168. M. Toyota, J.P. Issa (2000), *The role of DNA hypermethylation in human neoplasia.* Electrophoresis. **21**(2), 329-33.
169. S.B. Baylin and P.A. Jones (2012), *A decade of exploring the cancer epigenome — biological and translational implications.* Nat Rev Cancer **11**(10), 726–734. doi:10.1038/nrc3130
170. M. Esteller, J.M. Silva, G. Dominguez, F. Bonilla, X. Matias-Guiu, E. Lerma, E. Bussaglia, J. Prat, I.C. Harkes, E.A. Repasky, E. Gabrielson, M. Schutte, S.B. Baylin, J.G. Herman (2000), *Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors*. J Natl Canc Inst, **92**(7):564-569 **.**
171. R. Kanwal, S. Gupta (2010), Epigenetics and cancer. J Appl Physiol. **109**, 598–605.
172. P. A Jones and P. W Laird (1999), *Cancer-epigenetics comes of age*. Nature Genetics **21**, 163–167
173. M.M. Weil, J.S. Bedford, H. Bielefeldt-Ohmann, F.A. Ray, P.C. Genik, E.J. Ehrhart, et al. (2009), *Incidence of acute myeloid leukemia and hepatocellular carcinoma in mice irradiated with 1 GeV/nucleon (56)Fe ions.* Radiat Res; 172:213.
174. I. Koturbash, I. Pogribny, O. Kovalchuk (2005), *Stable loss of global DNA methylation in the radiation-target tissue—A possible mechanism contributing to radiation carcinogenesis?* Biochemical and Biophysical Research Communications **337,** 526–533.
175. J. Loree, I. Koturbash, K. Kutanzi, M. Baker, I. Pogribny, O. Kovalchuk (2006), *Radiation-induced molecular changes in rat mammary tissue: possible implications for radiation-induced carcinogenesis*. Int J Radiat Biol. **82**, 805–15.
176. D. Takai, P.A. Jones (2002), *Comprehensive analysis of CpG islands in human chromosomes 21 and 22.* Proc Natl Acad Sci U S A, **99**(6),3740-5.
177. M.F. Fraga, E. Ballestar, A. Villar-Garea, et al. (2005), *Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer*. Nat. Genet., **37**, 391–400.

178. D.B. Seligson, S. Horvath, T. Shi, H. Yu, S. Tze, M. Grunstein, S.K. Kurdistani. (2005), *Global histone modification patterns predict risk of prostate cancer recurrence*. Nature, **435**, 1262–1266.
179. K.B Reddy (2015), *MicroRNA (miRNA) in cancer*, Cancer Cell International **15**:38, doi:10.1186/s12935-015-0185-1.
180. Y. Peng, and C. M. Croce (2016), The role of MicroRNAs in human cancer. Signal Transduction and Targeted Therapy **1**, 15004; doi:10.1038/sigtrans.2015.4
181. D. Wang, J. Liu, T. Huo, Y. Tian, L. Zhao, (2017), *The role of microRNAs in colorectal liver metastasis: Important participants and potential clinical significances*. Tumor Biology, 1-10**,** https://doi.org/10.1177/1010428317709640
182. D. Li, Y. Ilnytskyy, A. Kovalchuk, L.M. Khachigian, R.T. Bronson, B. Wang, O. Kovalchuk (2013), *Crucial Role for Early Growth Response-1 in the Transcriptional Regulation of miR-20b in Breast Cancer.* Oncotarget **4**,1373-1387.
183. O.A. Kent, J.T. Mendell (2006), *A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes.* Oncogene **25**, 6188–96.
184. H. Osada, T. Takahashi (2007), *MicroRNAs in biological processes and carcinogenesis*. Carcinogenesis, **28**(1), 2-12.
185. L. He, X. He, L.P. Lim, E. de Stanchina, Z. Xuan, Y. Liang, W. Xue, L. Zender, J. Magnus, D. Ridzon, A.L. Jackson, P.S. Linsley, C. Chen, S.W. Lowe, M.A. Cleary, G.J. Hannon (2007), *A microRNA component of the p53 tumour suppressor network.* Nature, **447**(7148),1130–1134.
186. Q. Li, S. Li, Y. Wu, F. Gao (2017), *MiRNA-708 functions as a tumour suppressor in hepatocellular carcinoma by targeting SMAD3* Oncology Letters, **14**, 2552-2558.
187. J.B. Little. (2000). *Radiation carcinogenesis.* Carcinogenesis, 21(3):397-404
188. E.G. Wright (2000), *Inducible genomic instability: newinsights into the biological effects of ionizing radiation*, Med Conflict Survival, **16**, 117–130, discussion 131–113.
189. B.C. Christensen and C.J. Marsit (2011), *Epigenomics in environmental health*. Front. Genet., **2**:84 https://doi.org/10.3389/fgene.2011.00084.
190. B. Tanno, G. Babini, S. Leonardi, P. Giardullo, I. De Stefano, E. Pasquali, A. Ottolenghi, M.. J. Atkinson, A. Saran, M. Mancuso (2006), *Ex vivo miRNome analysis in Ptch1+/− cerebellum granule cells reveals a subset of miRNAs involved in radiation-induced medulloblastoma*, Oncotarget, **7**(42), 68253-69.
191. C. Liu, B. Li, Y. Cheng, J. Lin, J. Hao, S. Zhang, R.E. Mitchel, D. Sun, J. Ni, L. Zhao, F. Gao, J. Cai (2011), *MiR-21 plays an important role in radiation induced carcinogenesis in BALB/c mice by directly targeting the tumor suppressor gene Big-h3*. Int J Biol Sci. **7**(3), 347-63.
192. K. Kamiya, J. Yasukawa-Barnes, J. M. Mitchen, M. N. Gould, K. H. Clifton (1995) *Evidence that carcinogenesis involves an imbalance between epigenetic high-frequency initiation and suppression of promotion.* Proc. Natl. Acad. Sci. USA,. **92**: 1332-1336
193. K.H. Clifton (1996), *Comments on the evidence in support of the epigenetic nature of radiogenic initiation*. Mutation Research **350**:77-80
194. O. Kovalchuk, P. Burke, A. Arkhipov, N. Kuchma, S.J. James, , I. Pogribny (2003), *Genome hypermethylation in Pinus silvestris of Chernobyl – a mechanism for radiation adaptation ?* Mut Res **529**, 13-20.
195. I. Kovalchuk, V. Abramov, I. Pogribny, O. Kovalchuk (2004), *Molecular aspects of plant adaptation to life in the Chernobyl zone.* Plant Physiology **135**, 357-63.
196. S.A. Belinsky, D.M. Klinge, K.C. Liechty, T.H. March, T. Kang, F.D. Gilliland, N. Sotnic, G. Adamova, G- Rusinova, V. Telnov (2004), *Plutonium targets the p16 gene for inactivation by promoter hypermethylation in human lung adenocarcinoma*. *Carcinogenesis* **25**, 1063–1067.
197. A. Romanenko, L. Morell-Quadreny, J.A. Lopez-Guerrero, A. Pellin, V. Nepomnyaschy, A.Vozianov, A. Llombart-Bosch (2002), *P16INK4A and p15INK4B gene alteration associated with oxidative stress in renal cell carcinomas after the Chernobyl accident (pilot study)*. Diagn Mol Pathol. **11**(3):163-169.
198. C.M Wilke, J.Hess, S. V. Klymenko, V. V. Chumak, L.M. Zakhartseva, E.V Bakhanova, A. Feuchtinger, A. K..Walch, M. Selmansberger, H. Braselmann, L. Schneider, A.. Pitea, J. Steinhilber, F. Fend, H. C. Bösmüller, H. Zitzelsberger, K.. Unger (2018), *Expression of miRNA-*

26b-5p and its target TRPS1 is associated with radiation exposure in post-Chernobyl breast cancer. Int. J. Cancer, **142**: 573–583.
199. C. A. Meyers (2000), *Neurocognitive dysfunction in cancer patients*. Oncology **14**, 75–79, discussion 79, 81–72, 85.
200. F. A.Cucinotta, M. Alp, F. M. Sulzman, M. Wang (2014), *Space radiation risks to the central nervous system.* Life Sciences in Space Research **2**, 54–69.
201. V.K. Parihar, B. Allen, K.K. Tran, T.G. Macaraeg, E.M. Chu,S.F. Kwok, N.N. Chmielewski, B.M. Craver, J.E. Baulch,M.M. Acharya, F.A. Cucinotta, C.L. Limoli (2015), What happens to your brain on the way to Mars. Sci. Adv. **1**:e1400256.
202. S. Impey. T. Jopson, C. Pelz, A. Tafessu, F. Fareh, D. Zuloaga, T. Marzulla, L.-K. Riparip, B. Stewart, S. Rosi, M.S. Turker, J. Raber (2016), *Short- and long-term effects of 56Fe irradiation on cognition and hippocampal DNA methylation and gene expression*. BMC Genomics **17**:825, DOI 10.1186/s12864-016-3110-7.
203. M. M. Acharya, A.A. D. Baddour, T. Kawashita, B.D. Allen, A.R. Syage, T. H. Nguyen, N. Yoon, E. Giedzinski, L. Yu, V. K. Parihar, J.E. Baulch (2017), *Epigenetic determinants of space radiation-induced cognitive dysfunction* Scientific Reports **7**, Article number 42885, DOI: 10.1038/srep42885..
204. A.F. Trollope, K.R. Mifsud, E.A. Saunderson, J.M.H.M. Reul (2017), *Molecular and Epigenetic Mechanisms Underlying Cognitive and Adaptive Responses to Stress*. Epigenomes, **1**, 17; doi:10.3390/epigenomes1030017.
205. W.F. Morgan and W.J. Bair (2013), *Issues in Low Dose Radiation Biology: The Controversy Continues. A Perspective*. Radiation Research 179(5), 501-510, https://doi.org/10.1667/RR3306.1
206. D.T. Goodhead (1988), *Spatial and temporal distribution of energy.* Health Phys. **55**(2), 231-40.
207. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (1993), *Sources and Effects of Ionizing Radiation*. 1993 Report to the General Assembly, with Scientific Annexes, New York, 1993.
208. S.A. Amundson, K.T. Do, A.J. Jr Fornace (1999), *Induction of stress genes by low doses of gamma rays*. Radiat Res. **152**, 225–231.
209. Z. Goldberg, D.M. Rocke, C. Schwietert, S.R. Berglund, A. Santana, A. Jones, J. Lehmann, R. Stern, R. Lu, C. Hartmann Siantar (2006), *Human in vivo dose-response to controlled, low-dose low linear energy transfer ionizing radiation exposure*. Clin Cancer Res., **12**, 3723–3729.
210. A.J.Bernal, D.C, Dolinoy, D. Huang, D.A Skaar, C. Weinhouse, R.L. Jirtle (2013), *Adaptive radiation-induced epigenetic alterations mitigated by antioxidants*. FASEB Journal, **27**(2), 665-671. doi: 10.1096/fj.12-220350.
211. E. Fratini, C. Carbone, D. Capece, G. Esposito, G. Simone, M. A. Tabocchini, M. Tomasi, M. Belli, L. Satta (2015), *Low-radiation environment affects the development of protection mechanisms in V79 cells*. Radiat Environ Biophys DOI 10.1007/s00411-015-0587-4.
212. K. Taki, B. Wang, T. Nakajima, J. Wu, T. Ono, Y. Uehara, T. Matsumoto, Y. Oghiso, K. Tanaka, K. Ichinohe, S. Nakamura, S. Tanaka, J. Magae, A. Kakimoto, M. Nenoi (2009), *Microarray Analysis of Differentially Expressed Genes in the Kidneys and Testes of Mice after Long-term Irradiation with Low-dose-rate γ-rays.* J. Radiat. Res. **50**, 241-252.
213. S. Ye, D. Yuan, Y. Xie, Y. Pan, C. Shao (2013), *Role of DNA methylation in long-term low-dose γ-rays induced adaptive response in human B lymphoblast cells*. Int J Radiat Biol.; **89**(11):898-906. doi: 10.3109/09553002.2013.806832. Epub 2013 Sep 12.
214. J. L. García-Giménez, M. Seco-Cervera, T. O. Tollefsbol, C. Romá-Mateo, L. Peiró-Chova, P. Lapunzina, F. V. Pallardó (2017), *Epigenetic biomarkers: Current strategies and future challenges for their use in the clinical laboratory.* Critical Reviews in Clinical Laboratory Sciences **54**(7-8),1-22, doi 10.1080/10408363.2017.1410520.
215. E. Pernot, J. Hall, S. Baatout, M.A. Benotmane, E. Blanchardon, S. Bouffler, H. El Saghire, M. Gomolka, A. Guertler, M. Harms-Ringdahl, P. Jeggo, M.a Kreuzer, D. Laurier, C. Lindholm, R. Mkacher, R. Quintens, K. Rothkamm, L. Sabatier, S. Tapio, F.t de Vathaire, E. Cardis (2012), *Ionizing radiation biomarkers for potential use in epidemiological studies*. Mutation Research, **751**(2), 258-286.

216. T. Mikeska1, J.M. Craig (2014), *DNA Methylation Biomarkers: Cancer and Beyond*. Genes **5**(3), 821–864.
217. M.J. Hoffmann, W.A Schulz (2005), *Causes and consequences of DNA hypomethylation in human cancer.* Biochemistry and Cell Biology, **83**(3), 296-321
218. R Kanwala and S Gupta (2012), *Epigenetic modifications in cancer*. Clin Genet. **81**(4), 303–311.
219. C. Weigel, M.R. Veldwijk, C.C. Oakes, P. Seibold, A. Slynko, D.B. Liesenfeld, M. Rabionet, S.A. Hanke, F. Wenz, E. Sperk, A. Benner, C. Rösli, R. Sandhoff, Y. Assenov, C. Plass, C. Herskind, J. Chang-Claude, P. Schmezer, O. Popanda (2016), *Epigenetic regulation of diacylglycerol kinase alpha promotes radiation-induced fibrosis*. Nature Communications, DOI: 10.1038/ncomms10893
220. J. Li, X. Xing, D. Li, B. Zhang, D.G. Mutch§, I. S. Hagemann§, T. Wang (2017), *Whole-Genome DNA Methylation Profiling Identifies Epigenetic Signatures of Uterine Carcinosarcoma*. Neoplasia (2017) **19**, 100–111.
221. P. Costa-Pinheiro, D. Montezuma, R. Henrique, C. Jerónimo (2015), *Diagnostic and prognostic epigenetic biomarkers in cancer*. Epigenomics **7**(6),1003-15. doi: 10.2217/epi.15.56.
222. M.C. Myzak, R.H. Dashwood (2006), *Histone deacetylases as targets for dietary cancer preventive agents: lessons learned with butyrate, diallyl disulfide, and sulforaphane*. Curr Drug Targets. **7**(4), 443-52.
223. M.H. Barcellos-Hoff, C. Park, E.G. Wright (2005), *Radiation and the microenvironment - tumorigenesis and therapy* Nature Reviews Cancer **5**,867-875.
224. M. Tomasetti, W. Lee, L. Santarelli, J. Neuzil (2017), Exosome-derived microRNAs in cancer metabolism: possible implications in cancer diagnostics and therapy. Experimental & Molecular Medicine **49**, e285; doi:10.1038/emm.2016.153.
225. Guil S, Esteller M. (2009), *DNA methylomes, histone codes and miRNAs: tying it all together*. Int J Biochem Cell Biol. **41**(1):87-95. doi: 10.1016/j.biocel.2008.09.005.
226. M. Niyazi, F. Zehentmayer, O.M. Niemoller, S. Eigenbrod, H. Kretzschmar, K.S. Osthoff,. J,-C. Tonn, M. Atkinson, S. Mörtl, C. Belka (2011). *MiRNA expression patterns predict survival in glioblastoma.* Radiat Oncol 2011;**6**:153, doi:10.1186/1748-717X-6-153.
227. J. Hayes, P.P. Peruzzi, S. Lawler (2014), *MicroRNAs in cancer: biomarkers, functions and therapy.* Trends Mol Med. **20**(8),460-9. doi: 10.1016/j.molmed.2014.06.005.
228. M. Niyazi, A. Pitea, M. Mittelbronn, J. Steinbach, C. Sticht, F. Zehentmayr, D. Piehlmaier, H. Zitzelsberger, U. Ganswindt, C. Rödel, K. Lauber, C. Belka, K. Unger (2016), *A 4-miRNA signature predicts the therapeutic outcome of glioblastoma*. Oncotarget; **7**:45764-45775. doi.org/10.18632/oncotarget.9945.
229. S. Josson, S.-Y. Sung, K. Lao, L.W.K. Chung, P.A.S. Johnstone (2008), *Radiation modulation of MicroRNA in prostate cancer cell lines.* Prostate, **68**: 1599–1606. doi:10.1002/pros.20827
230. J. Ni, J. Bucci, L. Chang, D. Malouf, P. Graham, Y. Li (2017), *Targeting MicroRNAs in Prostate Cancer Radiotherapy.* Theranostics; **7**(13), 3243-3259.
231. T. Templin, S.A. Amundson, D.J. Brenner, L.B. Smilenov (2011), *Whole mouse blood microRNA as biomarkers for exposure to γ-rays and ^{56}Fe ions.* Int. J. Radiat. Biol., **87**, 653–662.
232. T. Templin, E.F. Young, L.B. Smilenov (2012), *Proton radiation-induced miRNA signatures in mouse blood: Characterization and comparison with ^{56}Fe-ion and gamma radiation*. Int J Radiat Biol. **88**(7): 531–539.
233. S. Grison, J.-C. Martin, L. Grandcolas, N. Banzet, E. Blanchardon, E. Tourlonias, C. Defoort, G. Favé, R. Bott, I. Dublineau, P. Gourmelon, M. Souidi (2012), *The Metabolomic Approach Identifies a Biological Signature of Low-dose Chronic Exposure to Cesium 137*. Journal of Radiation Research, **53**(1), 33–43, doi.org/10.1269/jrr.11071
234. L. Mirbahai, J.K. Chipman (2014), *Epigenetic memory of environmental organisms: A reflection oflifetime stressor exposures*. Mutation Research **764–765**,10–17.
235. MELODI (2017), Strategic Research Agenda of the Multidisciplinary European Low Dose Initiative (MELODI) - 2017. http://www.melodi-online.eu/doc/MELODI_SRA_2017_06102017
236. V.R Nelson and J.H Nadeau (2010), *Transgenerational genetic effects*. Epigenomics, **2**(6), 797–806. doi:10.2217/epi.10.57.

237. C.B. Yoo and P.A. Jones (2006), *Epigenetic therapy of cancer: past, present and future*, Nature Reviews Drug Discovery **5**, 37–50.
238. Y. Zhu, J. Hu, Y. Hu, W. Liu (2009), *Targeting DNA repair pathways: A novel approach to reduce cancer therapeutic resistance*. Cancer Treatment Reviews, **35**(7), 590 – 596.
239. K.M. Smits, V. Melotte, H.E.C. Niessen, L. Dubois, C. Oberije, E.G.C. Troost, M.H.W. Starmans, P.C. Boutros, M. Vooijs, M. van Engeland, P. Lambin (2014), *Epigenetics in radiotherapy: Where are we heading?* Radiotherapy and Oncology **111**, 168–177.
240. L. Stone (2016), *Prostate cancer: Radiotherapy induces epigenetic changes*. Nature Reviews Urology **13**, 241, doi:10.1038/nrurol.2016.68.
241. C. Peitzsch, M. Cojoc, L. Hein, I. Kurth, K. Mabert , F. Trautmann, B. Klink, E. Schrock , M.P. Wirth, M. Krause, E.A. Stakhovsky, G.D. Telegeev, V. Novotny, M. Toma, M. Muders, G.B. Baretton, F.M. Frame, N.J. Maitland, M.l Baumann, A. Dubrovska (2016), *An Epigenetic Reprogramming Strategy to Resensitize Radioresistant Prostate Cancer Cells*. Cancer Res; **76**(9), doi: 10.1158/0008-5472.CAN-15-2116.
242. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2000), *Sources and Effects of Ionizing Radiation. Volume II: Effects, 2000 Report to the General Assembly, with scientific annexes*. New York, United Nations, 2000.
243. D. Atri, A. L. Melott (2014), *Cosmic Rays and Terrestrial Life: A Brief Review*, Astroparticle Physics, **53**, 186-190, doi: 10.1016/j.astropartphys.2013.03.001.
244. N. Lampe, V. Breton, D. Sarramia, T. Sime-Ngando, D.G. Biron (2017), *Understanding low radiation background biology through controlled evolution experiments.* Evolutionary Applications, 10:658‑666, doi.org/10.1111/eva.12491.
245. F.A. Cucinotta and M. Durante (2006), *Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings.*Lancet Oncology, **7**(5), 431-435.
246. H. Planel, J. P. Soleilhavoup, R. Tixador, G. Richoilley (1976), *Demonstration of a stimulating effect of natural ionizing radiation and of very low radiation doses on cell multiplication*. In: IAEA, editor. Symposiumon biological effects of low-level radiation pertinent to protection of man and his environment. International Atomic Energy Agency, Vienna (Austria), 1976. p. 127-139.
247. H. Planel, J. P. Soleilhavoup, R. Tixador, G. Richoilley, A. Conter, F. Croute, C. Caratero,Y. Gaubin, (1987), *Influence on cell proliferation of background radiation or exposure to very low, chronic gamma radiation. Health Physics*, 52(5), 571–578.
248. G.B Smith, Y. Grof, A. Navarrette, R.A. Guilmette (2011), *Exploring biological effects of low background radiation from the other side of the background*. Health Physics, **100**(3), 263-265, https://doi.org/10.1097/HP.0b013e318208cd44.
249. H. Castillo, D. Schoderbek, S. Dulal, G. Escobar, J. Wood, R. Nelson, G. Smith (2015), *Stress induction in the bacteria Shewanella Oneidensis and Deinococcus Radiodurans in response to below-background ionizing radiation.* International Journal of Radiation Biology, **3002**, 1-33. https://doi.org/10.3109/09553002.2015.1062571.
250. Satta, L., Augusti-Tocco, G., Ceccarelli, R., Esposito, A., Fiore, M., Paggi, P., Poggesi I, Ricordy R, Scarsella G, Cundari, E. (1995), *Low environmental radiation background impairs biological defence of the yeast Saccharomyces cerevisiae to chemical radiomimetic agents*. Mutatation Research, **347**, 129–133. https://doi. org/10.1016/0165-7992(95)00031-3.
251. L. Satta, F. Antonelli, M. Belli, O. Sapora, G. Simone, E. Sorrentino,. M.A., Tabocchini, F. Amicarelli, C. Ara, S. Nisi (2002), *Influence of a low background radiation environment on biochemical and biological responses in V79 cells*. Radiation and Environmental Biophysics, **41**(3), 217–224. https://doi.org/10.1007/ s00411-002-0159-2.
252. M.C. Carbone, M. Pinto, F. Antonelli, F. Amicarelli, M. Balata, M. Belli, L. Conti Devirgiliis, O. Sapora, G. Simone, E. Sorrentino, M.A. Tabocchini, L. Satta (2010), *Effects of deprivation of background environmental radiation on cultured human cells.* Il Nuovo Cimento **4**,469–477
253. P. Morciano, R. Iorio, D. Iovino, F. Cipressa, G. Esposito, A. Porrazzo, L. Satta, E. Alesse, M. A. Tabocchini, G. Cenci (20018), *Effects of reduced natural background radiation on Drosophila melanogaster growth and development as revealed by the FLYINGLOW program*. J Cell Physiol; 233(1), 23-29.

254. S. Kurdyukov, M. Bullock (2016), *DNA Methylation Analysis: Choosing the Right Method*. Biology **2016**, 5, 3; doi:10.3390/biology5010003
255. O.C. Maes, J. An, H. Sarojini, H. Wu, E. Wang (2008), *Changes in MicroRNA expression patterns in human fibroblasts after low-LET radiation*. J. Cell. Biochem. **105**, 824–834.
256. D.F Durso, M.G Bacalini, I. Faria do Valle, C. Pirazzini, M. Bonafé, G. Castellani, A.M. Caetano Faria, C. Franceschi, P. Garagnani, C. Nardini (2017), *Aberrant methylation patterns in colorectal cancer: a meta-analysis.* Oncotarget, **8**(8), 12820–12830. http://doi.org/10.18632/oncotarget.14590
257. P.A. Jones, S.B. Baylin (2002), *The fundamental role of epigenetic events in cancer*. Nature Reviews Genetics 3, 415–428. doi:10.1038/nrg816.
258. A. Mezentsev and S.A. Amundson (2011), *Global Gene Expression Responses to Low- or High-Dose Radiation in a Human Three-Dimensional Tissue Model*. Radiat Res. **175**(6), 677–688.

XVIII CONVEGNO NAZIONALE
SIRR 2018
Università degli Studi Roma Tre, Roma, 10-13 Settembre 2018

La Società Italiana per le Ricerche sulle Radiazioni (SIRR) è lieta di presentare il XVIII Convegno Nazionale SIRR, che si terrà a Roma dal 10 al 13 settembre 2018 presso l'Università degli Studi Roma Tre. Nell'ambito del Convegno verranno presentati i più recenti studi nelle discipline proprie della Società (fisica, biologia, ingegneria, chimica, medicina) finalizzati al miglioramento delle conoscenze di base e della ricerca applicata in ambito biomedico, radioprotezionistico e industriale.

Topics

Meccanismi biologici radioindotti; Chimica delle radiazioni; Dosimetria delle radiazioni; Applicazioni cliniche delle radiazioni ionizzanti; Radioprotezione; Esposizioni a radiazioni cosmiche; Radiazioni non ionizzanti; Emergenze radiologiche e nucleari

Comitato organizzatore

Antonella Sgura; Valentina Dini; Francesca Antonelli; Mariagabriella Pugliese

Comitato scientifico

Mariagabriella Pugliese; Francesca Antonelli; Silva Bortolussi; Valentina Dini; Daniele Dondi; Maria Mirri; Rosa Sciuto; Antonella Sgura; Lidia Strigari

www.sirr2.it