



## **Telomere shortening and ionizing radiation: a possible role in vascular dysfunction?**

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### **Abstract**

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# TELOMERE SHORTENING AND IONIZING RADIATION: A POSSIBLE ROLE IN VASCULAR DYSFUNCTION?

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## ABSTRACT

**PURPOSE:** In recent years, growing epidemiological evidence linked ionizing radiation exposure to cardiovascular atherosclerotic disease. However, there are still major gaps in the knowledge of the molecular mechanisms of radiation-induced vascular disease, especially for low-dose levels. Telomeres, repetitive DNA sequences of (TTAGGG)<sub>n</sub> located at the ends of eukaryotic chromosomes, play a role in regulating vascular aging, and shorter leukocyte telomere length has been demonstrated to predict cardiovascular disease and mortality. There is also evidence supporting the crucial role of telomeres in the formation of chromosome and chromatid aberrations induced by ionizing radiation. **CONCLUSIONS:** The purpose of the present paper is to review the recent advances in the biological mechanisms determining telomere length erosion after ionizing radiation exposure as well as to examine the hypothesis that telomere shortening may be the crucial mediator leading to detrimental vascular effects after ionizing radiation exposure.

## INTRODUCTION

The high and unprecedented levels of ionizing radiation exposure in general population and exposed medical workers is a pivotal scientific and social problem (Center for Devices and Radiological Health, U.S. Food and Drug Administration, 2010). Medical radiation from X-rays and nuclear medicine is the largest manmade source of radiation exposure in western countries, accounting for a mean effective dose of 3.0 mSv per capita per year, similar to the risk of 150 chest X-rays (Picano 2004, Cone 2010). About 30 million workers are professionally exposed to radiation (United Nations Scientific Committee on the Effects of Atomic Radiation, UNSCEAR, Report 2008), and of these the interventional fluoroscopists (cardiologists and radiologists) are among the most exposed, with an annual exposure equivalent to 5 mSv per year, and a projected lifetime attributable excess cancer risk of 1 in 100 (Venneri et al. 2009).

Ionizing radiation is considered a non-threshold carcinogen, and the current risk estimates recommended for the radioprotection regulation are based on the assumption that all radiation exposures pose a risk in linear proportion to the dose (Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation 2006, Mullenders et al. 2009).

Furthermore, it is well established that moderate to high doses of radiation increase the occurrence in exposed individuals, also of non-cancer health effects, especially cardiovascular disease (Stewart and Fajardo 1971).

Recent epidemiological studies have documented the possibility of vascular and cardiovascular effects after exposure to radiation doses much lower than those normally associated with cardiac injury if the exposure dose is localized to the thorax or heart (Bhatti et al. 2008, Little et al. 2008). From current evidence, according to the International Commission on Radiological Protection (ICRP) 2011 an excess cardiovascular risk is proven with a threshold

acute dose of about 0.5 Gy (or 500 mSv) for both cardiovascular and cerebrovascular diseases (ICRP Statement on Tissue Reactions 2011).

Anyway, there is currently a problem of scientific acceptance of vascular dysfunction in relation to low-dose radiation, as there is no established suitable biological model (European Commission Scientific Seminar 2008).

Several mechanisms, including endothelial dysfunction, inflammation, alterations of coagulation and platelet activity may have a relevant role in radiation-induced vascular effects (Schultz-Hector et al. 2007).

Radiation predisposes to the formation of inflammatory plaque and endothelial cell loss, and pro-inflammatory changes in the microvasculature are thought to be the driving events leading to microthrombi and occlusion of vessels, reduced vascular density, perfusion defects and focal ischemia and resulting in the progression of myocardial cell death and fibrosis (Schultz-Hector et al. 2007). More recently, after dose- and time-dependent studies on functional and structural cardiac damage in a mouse model, it was postulated that radiation damage of capillary system can be considered the underlying cause of myocardial detrimental consequences of radiation exposure (Seemann et al. 2011). On the other hand, ionizing radiation exerts health risk through damage to cellular DNA producing oxidized bases, DNA strand breaks, DNA damage and chromosomal/chromatid aberration. Radiobiological research supports the idea that one of the most important effects of low radiation exposure is the induction of a persistent genomic instability. Genomic destabilization may be crucial in the onset of atherosclerotic events (Andreassi and Botto 2003), suggesting a direct synergistic association between radiation effects and pathogenetic changes independently from radiation. Of particular relevance for vascular dysfunction may be the DNA damage associated with critically shortened telomeres. In fact, researches carried out over the last 10 years have demonstrated the relevance of shortened telomeres in vascular disorders and prediction of cardiac events (Fuster and Andrés 2006, Andreassi 2008, Mainous and Diaz 2010). An activated DNA response pathway induced by both oxidative DNA damage and telomere dysfunction is believed to be the crucial mediator for increased premature vascular aging and/or apoptosis, playing a pathogenetic role in atherosclerosis (Andreassi 2008). There is also evidence supporting the crucial role of telomeres in the formation of chromosome and chromatid aberrations induced by ionizing radiation (Shjepcevic et al. 1998). Anyway, a link between telomere shortening and vascular effects of ionizing radiation is still lacking.

The purpose of the present paper is to review the recent advances in the biological mechanisms determining telomere length erosion after ionizing radiation exposure as well as to examine the hypothesis that telomere shortening may be the crucial mediator leading to detrimental vascular effects of radiation ionizing exposure.

## **TELOMERE LENGTH TISSUE VARIABILITY: THE LEUKOCYTE MODEL**

Telomeres are evolutionary conserved repetitive deoxyribonucleic acid (DNA) sequences that “cap” the ends of the chromosomes (Blackburn 2001)

preventing the activation of DNA damage response. Telomeres are arranged in loop structures (T- and D- loops) and their length varies among species, in humans telomeres are composed of TTAGGG arrays up to 20 kilo-base pairs, terminating in a 3' single stranded DNA overhang made of 100-400 nucleotides (Figure 1) (Baird 2005). Telomeres have been defined as natural double-strand breaks (DSB) since they are specialized structures with the crucial role to distinguish normal ends from DSB, acting in conjunction with a highly organized system of proteins in the telomere-shelterin complex. Telomere length decreases at each cell division, and after a critical level of telomere shortening, cells lose the ability to replicate and may stop dividing (senescence) or entering apoptosis (Wong and Collins 2003). In normal somatic cells, the loss of genetic material is known as "end-replication problem" and consists in the inability of DNA polymerase to completely replicate the chromosomal termini, thus triggering replicative senescence (Figure 2) (Oeseburg et al. 2010). On the contrary, in germ cells telomere length is fully maintained by the telomerase (Figure 3). The length of telomeres is widely considered as a marker for biological aging, both at cellular and organism levels, as well as a potential marker of age-related diseases (Oeseburg et al. 2010). Even though the molecular and physiological mechanisms of telomere dynamics are not totally understood, it seems possible to believe that cells developed an accurate strategy first of all to discriminate between normal and altered telomeres and, secondarily, to keep a balance between the processes of telomere maintenance and repair (Butt et al. 2010).

Human telomere length varies between tissues from 12 to 16 kilobase. Furthermore, it may vary among chromosomes of the same cell and individuals of the same age (Rufer et al. 1999, Takubo et al. 2002). During fetal life, however, leukocyte telomere length appears to be very similar to the majority of tissues. In adulthood, the different cell proliferation rates induces a variability in the rate of telomere attrition in different tissues determining a 30-150 bp loss at each cell division (Harley et al. 1990, Kammori et al. 2002).

Telomere length has been studied in different tissues and shows a considerable variety from tissue to tissue, especially because of the difference in the cell turnover rate. Interesting data were obtained from a study performed in three different cellular types (leukocytes, skin cells and synovial cells) of nine elderly patients (Friedrich et al. 2000). Telomere measurements were obtained by telomere restriction fragment analysis and, independently of the age of the subjects, telomeres were significantly shorter in fast replicating cells (leukocytes) if compared to slower replicating cells (skin and synovium). However, a strong linear correlation was found in the telomere length between two of the three tissues at a time (Friedrich et al. 2000). These results suggest that genetic regulation of telomere length is independent from the tissue and that the telomere measurement in leukocytes could be an alternative parameter for the relative telomere length in other healthy tissues (Friedrich et al. 2000, von Zglinicki et al. 2000). Indeed, leukocytes are promptly available and easy to handle and these characteristics make them a suitable cell model for studying telomere attrition especially in particular conditions, such as for the study of tumor development, where it is crucial an intra-individual comparison with

healthy tissue from the same subject (Murnane and Sabatier 2004). The discover of leukocytes as suitable model in the study of telomere dynamics allowed a remarkable progress in human telomere research, triggering the discipline of telomere epidemiology (Wentzensen et al. 2011).

## **KEY ELEMENTS IN TELOMERE LENGTH REGULATION**

The maintenance of telomere length is a multifactorial process involving different mechanisms acting in a synergistic mode, depending mainly on the complex equilibrium regulating the association of telomeric sequences and specific proteins forming a high order regulating structure (Blackburn 2001, Aviv 2011).

### **Telomerase complex**

Telomerase function is one the principal factors in the maintenance of telomere integrity. Telomerase is a ribonucleoprotein complex formed by RNA and protein components, such as telomerase reverse transcriptase (Tert), a telomerase RNA component (Terc) and the stabilizer protein dyskerin (Cong et al. 2002, Cohen et al. 2007). Telomerase is highly expressed during the intrauterine life but its expression is markedly reduced or totally inhibited within a few weeks after birth in almost all somatic cells (Figure 3) (Serrano and Andres 2004). However, even in adult life, telomerase is up-regulated in rapid expanding cells such as lymphocytes, germ or stem cells where it plays a determinant role for the organism correct function (Marrone et al. 2004, Flores et al. 2006). Mutations in the telomerase core components, Tert and Terc, are typical hallmarks of “aplastic anemia” and “dyskeratosis congenital”. Both diseases are characterized by skin abnormalities and bone marrow failure, and dyskeratosis is characterized by an alteration of the hematopoietic stem cell compartment (Flores et al. 2005).

### **Telomeric chromatin**

Increasing evidence indicate also that telomere maintenance is under regulation of telomere-binding proteins and specific chromatin modification at telomeres, such as methylation of H3 at lysine 9 (H3K9me3) and H4 at lysine 20 (H4K20me3), which are characteristic of constitutive heterochromatin domains involved in the binding of isoforms of heterochromatin protein (HP1) (Blasco 2007). Interestingly, loss of these heterochromatic markers at telomeres leads to a less compacted chromatin and to excessively elongated telomeres, suggesting the presence of a higher-order control of telomere length by the state of telomeric chromatin (Schoeftner and Blasco 2009). The current more plausible view is that the function of telomerase at the chromosome ends is controlled by a balance between the direct molecular interactions of the enzyme at the telomere and the negative feedback process that regulates telomeres within a specific length range. In support of this hypothesis, there is the

evidence that telomerase does not elongate all telomeres indifferently at the same time but first selects the shortest ones (Blackburn 2001).

## TELOMERE SHORTENING AND VASCULAR DISEASE

During the last decade, many studies have linked telomeres and associated proteins to several factors which influence cardiovascular risk (eg, hypertension, diabetes, estrogens, oxidative stress and psychological stress), neovascularization, atherosclerosis and heart disease (Edo and Andrés 2005; Wong et al. 2008).

There is evidence indicating that telomere shortening occurs in human vessels, and this may be related to age-associated vascular disease (Minamino and Komuro 2007). Studies on telomere in the endothelial cells of the abdominal aorta and iliac arteries show a strong inverse correlation between telomere length and age (Chang and Harley 1995, Aviv et al. 2001). Moreover, in leukocytes from healthy subjects was observed an inverse correlation between the telomere length and the pulse pressure, that is independent of the chronological age (Benetos et al. 2001), while in patients with severe coronary artery disease, the telomeres of leukocytes are significantly shorter than those of cells from controls, which might reflect accelerated biological aging of various tissues (including the coronary arteries) (Benetos et al. 2004, Kurtz et al. 2006). Shorter telomeres are associated with an increase of carotid atherosclerosis in hypertensive subjects, whereas calcific aortic valve stenosis is correlated with telomere shortening in the elderly. Furthermore, experimental studies on neovascularization (Franco et al. 2002) reported an important impairment in the telomerase-deficient mice: the incapability to form new vessels may be due to the impairment of the replicative function of vascular endothelial cells induced by telomere shortening. Aging-related endothelial dysfunction seems to be a crucial factor correlating aging to cardiovascular disease, strongly associated with classical inflammatory risk markers. Therefore, endothelial dysfunction connected to atherogenic events (e.g., hypertension, diabetes, smoking) is determinant in the progression of atherosclerosis (Kurtz et al. 2006). The initial event may be a continuing mechanical or functional lesion to the endothelium. Injuries result in a locally increased cellular turnover enhancing the development of atherosclerotic plaques. As a consequence of augmented replicative events, human vascular endothelial cells undergo early cellular senescence, associated with telomere shortening (Balasubramanyam et al. 2007). Telomere attrition is higher in coronary artery endothelial cells from patients with coronary heart disease (CHD) when compared to healthy subjects. Independently of age, telomeres may be involved in the onset and progression of CHD. The leukocyte model in these type of studies is very useful since it does not reflect the telomere dynamics in altered tissues. Even though the distribution of CHD in the human population can be explained through various specific cardiovascular risk factors, coming to single individuals there is an high variability among subjects with analogous clinical profiles. The more reliable explanation is that such variability might be attributable to the different biological aging rate (Balasubramanyam et al. 2007). The fact that telomere shortening associated to

CHD is often irrespective of common risk factors of CHD, the hypothesis that telomere shortening might be a consequence of the progression of the disease can not be ruled out and more investigations need to be done in this direction. An interesting hypothesis postulates that the association of shorter telomere with the level of CHD risk may have a genetic basis (Slagboom et al. 1994). The intriguing possibility that subjects with shorter telomeres at birth might have a higher probability to develop CHD, opens a new scenario on telomere potentials as biological marker and predictor of cardiovascular-related disease. In fact, the important implication of this “genetic” hypothesis is that, if true, the shorter telomeres in leukocytes (and by inference other tissues) change from simply being a marker of the atherosclerotic process and become a primary alteration, which provides a substrate for more rapid biological aging and cellular senescence in response to the presence of other atherosclerotic risk factors (Samani et al. 2007).

### **IONIZING RADIATION AND DNA-DAMAGE RESPONSE AT TELOMERES**

DNA-damage response has evolved to optimize cell survival and to properly restore cell proliferation. The most conserved approach for cellular repair is the recruitment of specific DNA proteins to the altered DNA and the establishment of checkpoint events aimed to slow down or arrest cell-cycle progression, until the damage has been corrected (Zhou and Elledge 2000, Khanna and Jackson 2001). Upon DNA repairing, the blocks of cell-cycle are removed and cell proliferation resumes. Sometimes, the inability to repair damaged DNA and the excessive checkpoint activation can lead to an apoptotic outcome (Rich et al. 2000). DNA-damage checkpoint events seem very similar to typical cellular signal transduction pathways. Therefore, the DNA damage is detected by a specific DNA-damage-binding protein that leads to the activation of a kinase cascade that amplifies the initial DNA-damage signal and targeting various specific effectors. The main components of DNA damage response in all organisms studied so far belongs to the PIKK (phosphatidylinositol 3-kinase-like kinase) family. One of the most reliable indication for a crucial role of DNA-damage checkpoint factors at telomeres is that inactivation of checkpoint PIKK leads to unstable telomere length regulation. Thus, checkpoint PIKK activity is required for adequate telomere homeostasis, and the mechanism by which this occurs is strongly associated to an effective DNA damage response (d’Adda et al. 2004). Since a wide variety of DNA damage responses are involved in telomere length regulation, it is critical to evaluate the possibility that telomere maintenance could be an integral part of a complex system that acts at a more general level in the control of restoration of DNA sequence integrity and fidelity, rather than an independent mechanism (Ayouaz et al. 2008). As a consequence, the term “integrative” model indicates the telomere length preservation as an integral part of DNA damage response (Slijepcevic 2006). Ionizing radiation is a potent inducer of DNA-DSB and DSB occurring near telomeres would likely promote telomere loss. DSB, if unrepaired, may either lead to cell death or induce neoplastic transformation. Studies in mammals and yeast have shown that mutations in genes responsible for DNA-DSB repair



result in increased ionizing radiation sensitivity. Non-homologous end-joining (NHEJ) represents the main repair mechanism working throughout the cell cycle. The other DSB repair pathway, homologous recombination (HR), typically takes over in the S- and G2-phase when sister chromatids operate as templates for the repair machinery (Figure 4) (Shrivastav et al. 2008). The prevalent idea is that the two systems seem to act cooperatively more than competing, in fact, the arrest of one pathway is compensated by the intensified activity of the other. NHEJ represents the mechanism that rapidly repairs all DSB. Therefore, if some breaks are not promptly repaired, NHEJ is interrupted and DSB repair is performed by HR (Jeggo et al. 2011). Telomere length has been demonstrated to influence the cellular response to ionizing radiation. Different studies performed with a telomerase-deficient mouse model, null for telomerase RNA gene *mTerc*, demonstrated that short telomeres in late-generation *mTerc*<sup>-/-</sup> mice determine a condition of hypersensitivity of the mouse to ionizing radiations and a decreasing life span (Goytisolo et al. 2000, Wong et al. 2000). The major finding was that late generation *mTerc*<sup>-/-</sup> mice with short telomeres were more prone to accumulate chromosome aberrations when compared to early generation *mTerc*<sup>-/-</sup> or wild-type mice. No data are so far reported in humans on the effects of telomere attrition in the correct repair of DSB on radiosensitivity associated to ageing. Interestingly, in the work of Latre et al. (Latre et al. 2003), it was hypothesized that, in irradiated *mTerc*<sup>-/-</sup> null mice, the major mechanism for the chromosomal sensitivity is the presence of several chromosomes with eroded telomeres that, joining with the broken ends formed after irradiation, interfere with the appropriate repair mechanism preventing the correct restoration of the chromosome. Thus, short telomeres increase cell radiosensitivity and radiosensitive cells have shorter telomeres than normal cells. Interestingly, medical professional chronically exposed to relatively high doses of radiation (5 mSv/year) have increased levels of apoptosis and oxidative stress (Russo et al. 2012).

However, data from *in vivo* studies on telomere status after exposure to ionizing radiation in humans at the moment are very scarce. In a study on natural background radiation, Das et al. evaluated the possible long term detrimental effects of chronic low-dose radiation on human population and no significant correlation between level of radiation exposure with telomere length was found (Das et al. 2009). Moreover, in a study performed on elderly that have been exposed to ionizing radiation for professional reasons, it was demonstrated that telomere stability correlates with longevity (Sharma et al. 2003). On the other hand, in cancer patients undergoing radiotherapy, defective G2 chromosome repair and telomere erosion are strongly associated with the radiotherapeutic treatment (Sharma et al. 2003).

Finally, a recent study showed a significant telomere shortening in peripheral blood samples from Chernobyl clean-up workers both in the early and in the late period (even 20 years) after low-dose irradiation and these changes are related to variation in the apoptosis rates (Ilyenko et al. 2011).

Anyway, new insights in the complex dynamics regulating the telomere length and telomerase activity in relation to human ionizing radiation exposure are needed to better understand many aspects of human health and disease,

from cancer to non-cancer diseases, including vascular effects. In particular, the chronic effects of moderate (100 to 1000 mSv) exposure in fractionated doses to radiation remains unknown. This is also relevant in view of the emerging evidence that ionizing radiation can induce telomere shortening in acute high doses, and telomere shortening per se can increase radiation sensitivity (Drissi et al. 2011).

## IONIZING RADIATION AND OXIDATIVE STRESS

Even though the process by which telomere shortening in cells after ionizing radiation exposure is not well define yet, oxidative stress may play an important role in this attrition. In fact, the biological effect of ionizing radiation exposure is mainly caused by the formation and harmful actions of free radicals and reactive oxygen species (ROS). ROS can induce a large variety of DNA damage, including DNA strand breaks and DNA single copy modifications and, in particular, ROS promotes telomere shortening during the cell replication (von Zglinicki 2002). Oxidative damage to telomeric DNA is characterized by the synthesis of adducts of guanine, the 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), which are important as trigger for the disturbance of maintenance of chromosome length. In fact, because of the high content of guanines, the telomeres are critically sensitive to oxidative guanine damage (von Zglinicki et al. 2005, Wang et al. 2010). If these guanine lesions remain unrepaired, they become highly mutagenic leading to G:C to T:A transversion very rapidly. Mice lacking of the repair gene 8-oxoguanine DNA glycosylase (OGG1) are characterized by a high tumor predisposition (Xie et al. 2004) and Ogg1 null mice attenuates telomere integrity throughout the inability of DNA base excision repair activity (Wang et al. 2010).

Moreover, ROS can cause single strand breaks (SSB), acting directly or as part of the single base modification repair process. Unlike the majority of genomic DNA, telomeric DNA was reported to be unable to repair single-strand breaks. For this reason, telomeres appear to be a sensible target for ROS induced DNA-strand breaks (von Zglinicki et al. 2000). Furthermore, it has been hypothesized that the DNA damaged by ROS might interfere with the replication fork, thus increasing the telomere erosion (von Zglinicki 2002).

Oxidative stress seems to be the principal cause of telomere shortening in cultured cells (von Zglinicki et al. 2003) and hyperoxia and mitochondrial ROS induced-dysfunction determine an intense telomere attrition reducing proliferation of human cells in vitro (von Zglinicki et al. 2003). Interestingly, these effects are less evident if cells are grown in hypoxia or in the presence of antioxidant. Moreover, studies on human fibroblasts showed that even though long telomeres do not affect sensitivity to ionizing agents such as etoposide or bleomycin, they strongly sensitize cells to hydrogen peroxide, suggesting that telomeres are particularly vulnerable to oxidative aggression (Rubio et al. 2004).

Remarkable evidence of a correlation between oxidative stress and telomere shortening was found in epidemiological studies that investigated the association between telomere length and illnesses where oxidative stress and

inflammation are chronic conditions, such as in coronary atherosclerosis, myocardial infarction, hypertension, diabetes, obesity, lung diseases and even psychological pathologies (Benetos et al. 2001, Tezcan et al. 2003, Benetos et al. 2004, Adaikalakoteswari et al. 2005). Furthermore, various in vitro studies showed that chronic hypoxia preserves telomere length and extends the life span of cells, through an increase of telomerase activity (Haendeler et al. 2004, Minamino et al. 2001). One hypothesis is that Nitric Oxide (NO) might delay endothelial cell senescence interfering with telomerase activity and modulating the shortening of telomeres, probably reacting with oxygen radicals and reducing the oxidative stress (Vasa et al. 2000). Furthermore, senescence induced by oxidative stress in vascular endothelial cells seems to be associated with altered expression of various mitochondrial genes (Kumazaki et al. 1998). In general, oxidative damage to mitochondrial DNA (mtDNA) in somatic cells may produce various nucleotide modifications causing important mutations at mtDNA level. Consequently, the accumulation of mtDNA mutations may determine respiratory chain alteration, resulting in an increased generation of mitochondrial ROS which, in turn, causes further mtDNA mutations. This vicious cycle will progressively exhaust the efficiency of the mitochondrial function (Figure 5) (Wei et al. 2009). Interestingly, it has been demonstrated that mitochondrial integrity reduces with aging and that mtDNA naturally accumulates mutations (Balaban et al. 2005). The main aging-associated mutation of mtDNA is the wide common 4977 bp deletion (mtDNA<sup>4977</sup>) which is considered a biomarker of aging progression and mitochondrial impairment. Interestingly, various studies have underlined the importance of this deletion as a very sensitive marker of oxidative damage of mtDNA, because of the amplification effect of this mutation during mtDNA replication (Prithivirajasingh et al. 2004). Moreover, differently from the hardly detectable single copy deletions in mtDNA after radiation exposure, the much wider common deletion is more easily traceable, resulting in a very sensitive marker of mtDNA damage. Even though mtDNA<sup>4977</sup> represents only a fraction of the mtDNA damage following irradiation, it can be very representative for actual total damage (Prithivirajasingh et al. 2004, Wang et al. 2007). Presumably, radiation damage to mitochondrion may induce an increased production of ROS, playing a central role in cellular senescence (Passos and von Zglinicki 2005), increasing telomere shortening and accelerating the progression of vascular senescence (Figure 5). The relevance of oxidative stress in indirectly mediating the radiation effects on telomeres length is also supported by in vivo studies providing clear evidence of a bystander effect on telomere length at 0.5 Gy, but not at 0.1 Gy. With moderate 0.5 Gy dose, the toxic effect of radiation on telomeres can be reproduced by transferring the irradiated medium to unirradiated cells (Belloni et al. 2011).

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In summary, telomeres are efficient indicators of global exposition of the body to inflammatory and oxidative stimuli. Accumulating data demonstrate that oxidative stress produces increased erosion at telomeric ends resulting in

telomere length shortening which, in turn, leads to chromosome aberrations and genomic instability. In recent years, a large body of evidence showed that progressive telomere shortening is an important factor in the pathobiology of vascular disease, including metabolic syndrome, diabetes mellitus, coronary heart disease and premature myocardial infarction. Therefore, a plausible hypothesis is that telomere dysfunction may represent an important mediator between radiation exposure, ROS formation and vascular damage, as summarized in Figure 6.

Additional studies are critically needed in order to clarify if radiation-induced telomere shortening impacts on the development and progression of vascular dysfunction. In particular, we need to obtain well characterized dosimetric data to separate effects occurring in three distinct settings: 1) acute low-doses (<100 mSv) most frequently found in medical single test exposures, 2) moderate doses (100-1000 mSv), relevant for repetitive fractionated medical exposures and cumulative professional medical exposures, 3) and high doses (>1000 Sv) directly relevant to radiotherapeutic applications. Therefore, planned studies of radiation-exposed cohorts (e.g. professional exposed personnel, patients undergoing periodical therapeutic radiation treatment for non-malignant diseases, etc.) and new laboratory investigations (e.g. inflammatory and genetic marker analysis on leukocytes, vascular samples and cultured vascular endothelial cells) are needed to explore biological mechanisms of low-dose radiation-associated cardiovascular disease.

This topic is now on the top of scientific agenda of cardiologic and radiologic societies (Picano and Vano 2011). In fact, cardiac catheterization procedures increased from 2.45 millions in 1993 to 3.41 millions in 1997 and to 4.6 millions in 2006 in the USA (Mettleer et al. 2009) and similar trends were observed in Europe (Picano and Vano 2011). Each procedure involves a large radiation exposure of the patient, which may range from 7 to 57 mSv or more (average reference dose of 15 mSv), corresponding to an average dose of 750 mSv (350–2800 mSv) chest X-rays for percutaneous coronary intervention or cardiac radiofrequency ablation. Interventional cardiologists have to work close to the source of X-rays, and this explains why their own professional exposure is three times higher than that of radiologists (Picano and Vano 2011). Interventional cardiologists are part of a larger population of about 23 million people (excluding military personnel) professionally exposed to ionizing radiation worldwide. Therefore, interventional cardiologists and radiologists (and nurses and technicians working in the cath lab) are our clinical model in order to investigate the role of chronic low-dose radiation exposure in the etiology of vascular disease.

The ongoing Healthy Cath Lab study, organized by the Italian National Research Council with endorsement of Italian Society of Invasive Cardiologists (webcite: [http://www.gise.it/news/545/healthy\\_cath\\_lab\\_al\\_gise\\_2011\\_un\\_pr](http://www.gise.it/news/545/healthy_cath_lab_al_gise_2011_un_pr)) will precisely investigate the potentially increased health risks (in terms of atherosclerotic, reproductive and neurocognitive effects) of chronic low-dose radiation exposure. As an alternative to the epidemiological approach, the Healthy Cath Lab study will assess clinical health effects by early warning signs, which evaluate initial damage through easy to measure and non-invasive

surrogate endpoints, able to identify long-term risk for subsequent clinically overt disease, such as telomere length for atherosclerosis and aging.

In addition, bench model will investigate the deregulation of many aspects of vascular endothelial biology including inflammatory and pro-coagulant activation, cell growth, cell cycle control, and cell death as well as the regulation of cellular epigenetic marks.

The integrated clinical and bench approach will allow to better understand the role of telomere dysfunction in the peripheral blood cells and vascular cells after chronic low-dose exposure as well as to define whether telomere shortening may represent a new and powerful biomarker of radiation exposure.

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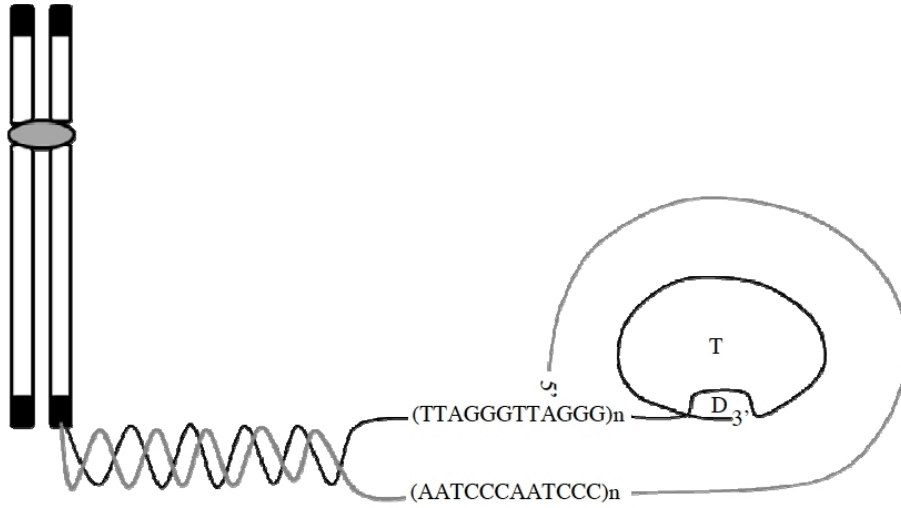
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JUST ACCEPTED

## Figure Legends

### Figure 1

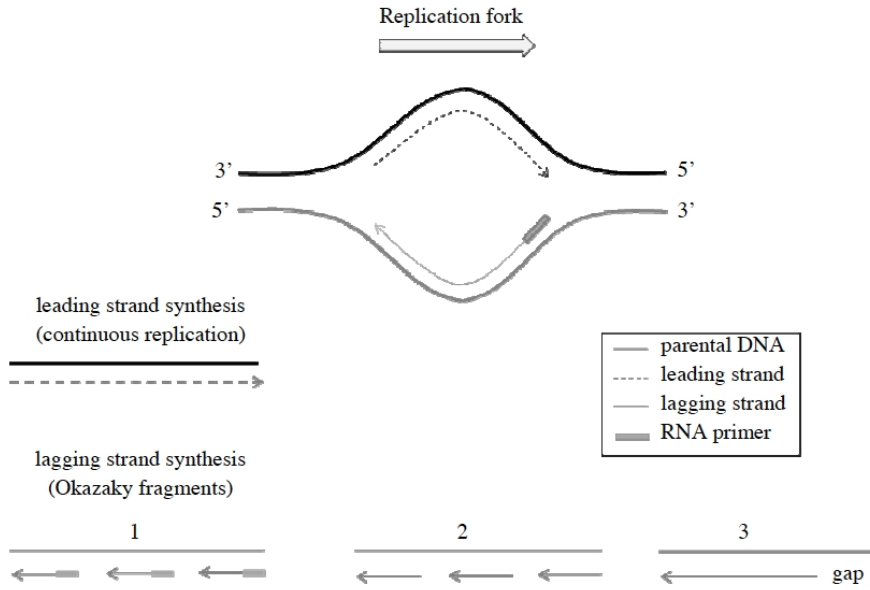
Simplified representation of a telomere folded in loop structures (T- and D-loops). Telomeres are located at the ends of the chromosomes and consist of (TTAGGG) $_n$  repeats.



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**Figure 2**

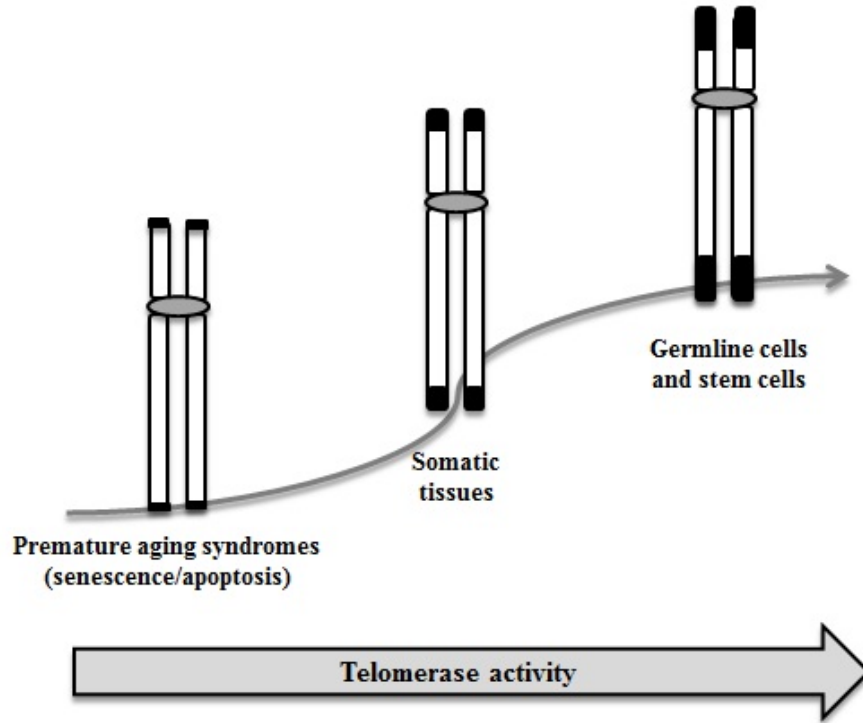
The “end replication problem” in telomere replication. DNA polymerase synthesizes in the 5'→3' direction. The leading strand is continuously synthesized while the lagging strand is synthesized from DNA fragments from primer sequences. Once primers are removed, the gaps at the telomere are not repaired.



JUST ACC

**Figure 3**

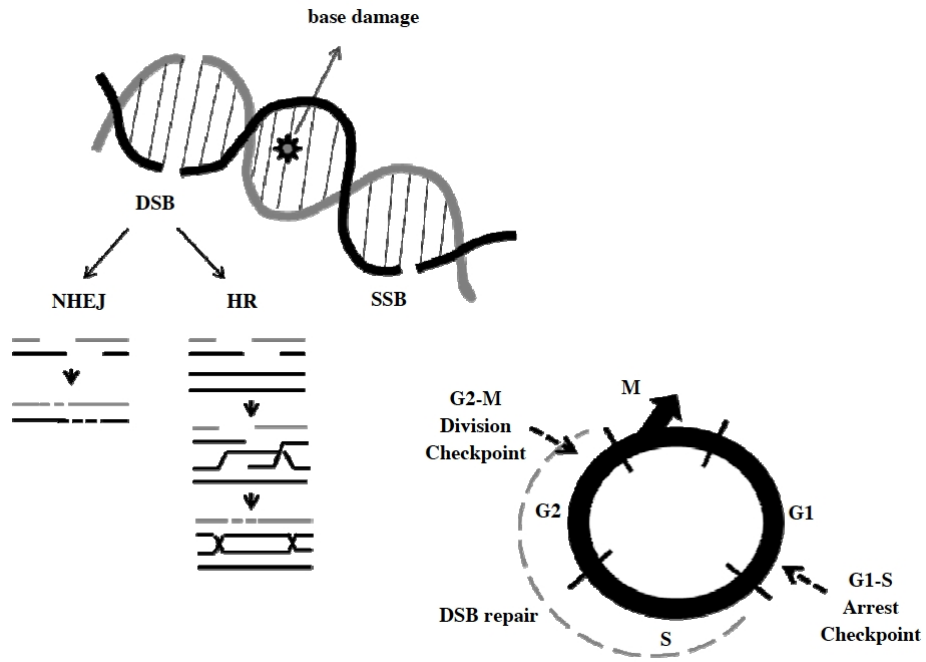
Telomere dynamics in human cells. Germ line and stem cells have high telomerase activity and maintain telomere length. In normal somatic cells, telomerase activity is low or absent and progressive telomere shortening occurs at each mitotic cycle. Telomere erosion is also observed in various human premature aging syndromes.



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**Figure 4**

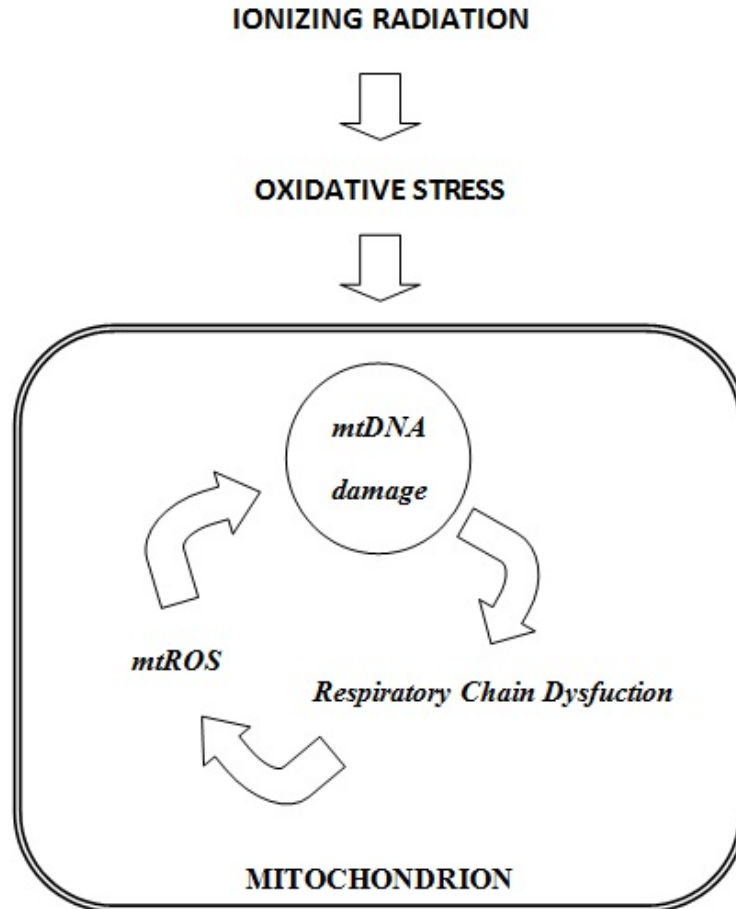
DNA damage response after ionizing radiation exposure. Double strand break (DSB) lesions induce signaling cascades leading to the activation of one of the two main repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). SSB = single strand break.



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**Figure 5**

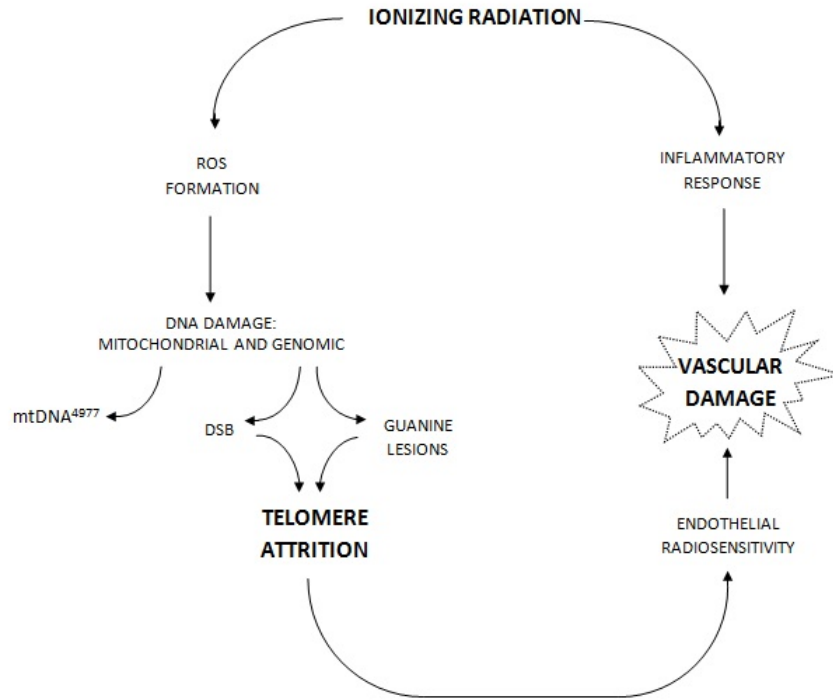
Somatic cell “vicious cycle” of mitochondrion response to ionizing radiation exposure: oxidative stress produces important mutations at mtDNA level that, in turn, may determine respiratory chain dysfunction leading to an increased production of ROS in mitochondria and induction of further mtDNA mutations. This vicious cycle will progressively exhaust the functional capacity of mitochondria.



JUST

**Figure 6**

Schematic representation of the ionizing radiation and telomere attrition hypothesis: telomere dysfunction may represent an important mediator between radiation exposure, ROS formation and vascular damage.



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