

RAYs STING: THE ACUTE CELLULAR EFFECTS OF IONIZING RADIATION EXPOSURE

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Abstract - High-precision radiation therapy is a clinical approach that uses the targeted delivery of ionizing radiation, and the subsequent formation of reactive oxygen species (ROS) in high proliferative, radiation sensitive cancers. In particular, in thoracic cancer radiation treatments, can not avoid a certain amount of cardiac toxicity. Given the low proliferative rate of cardiac myocytes, research has looked at the effect of radiation on endothelial cells and consequent coronary heart disease as the mechanism of radiation induced cardiotoxicity. In fact, little is known concerning the direct effect of radiation on mitochondria dynamics in cardiomyocyte. The main effect of ionizing radiation is the production of ROS and recent works have uncovered that they directly participates to pivotal cell function like mitochondrial quality control. In particular ROS seems to act as check point within the cell to promote either mitochondrial biogenesis and survival or mitochondrial damage and apoptosis. Thus, it appears evident that the functional state of the cell, as well as the expression patterns of molecules involved in mitochondrial metabolism may differently modulate mitochondrial fate in response to radiation induced ROS responses. Different molecules have been described to localize to mitochondria and regulate ROS production in response to stress, in particular GRK2. In this review we will discuss the evidences on the cardiac toxicity induced by X ray radiation on cardiomyocytes with emphasis on the role played by mitochondria dynamism.

Keywords: *Reactive Oxygen species; signal transduction; ionizing radiations, Mitochondria*

I. INTRODUCTION

Ionizing irradiation is defined as the transport of energy through the space. In biomedicine the effects

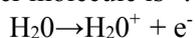
of irradiation are studied to evaluate the modifications on cells and tissues. Ionizing irradiation for example is a cause of cancer by inducing modifications of the genetic information in individual cell^{1,2}. At the same time, radiation is also applied for treatment of cancer with purpose of killing cancer cells³. About 60% of all cancer diseases are cured by radiotherapy alone or in combination with surgery⁴. The use of radiation in tumor therapy represents a compromise between maximal damage of tumor cells and the minimal deleterious effects for healthy tissues. For this purpose, high-precision radiation therapy has been developed to minimize damage of the surrounding normal tissues^{5,6}. This approach uses the delivery of ionizing radiation with selective formation of reactive oxygen species (ROS) in the targeted tissue⁷, a biological effect that can be relived starting within milliseconds after exposure. The specific subcellular alterations induced by radiation involve mainly plasma and mitochondria membrane with following increased production of ROS and possibly alteration of the mitochondrial function^{8,9}. However, even though this intensity-modulated radiotherapy can reduce the exposure to the normal tissues, a certain amount of radiation is still delivered in the area surrounding the neoplasia. The consequence of this damage can be divided in early and late reactions, based on their occurrence within hours (acute exposition)¹⁰ or days/months/years after therapy (chronic exposition)¹¹. The effects of ionizing radiation used in radiotherapy on different tissues are of a particular interest for the clinical consequences at the cardiovascular system. Thoracic radiotherapy is among the most frequent applications used for treatment of mediastina neoplasia, such as breast cancer or Hodgkin Lymphoma, and it is frequently associated to a clinically relevant cardiac toxicity, occurring as late reactions¹².

Several studies have pointed out the effects of radiation on vascular endothelial cells¹³⁻¹⁵ but recently it has been observed that radiation can also directly affects the cardiomyocytes¹⁶ and other cardiac structures leading to cardiomyopathy¹⁷, valves heart disease and conduction abnormalities¹⁸. However, the knowledge about the direct effects of radiation on the myocardium is still poor, as related to the effects on the single cardiomyocyte and the specific molecular alteration produced¹⁹. Mitochondria are considered the cardiomyocytes powerhouse and are at the same time the major source of ROS²⁰. Considering the relevance of mitochondria for cardiac functions, it is possible to speculate that the deleterious effects of a chronic irradiation could relate to the dysfunction of this organelle²¹. In this review we will discuss the latest evidences on the cardiac toxicity induced by ionizing radiation (X-ray) on cardiomyocytes with emphasis on the role played by mitochondria.

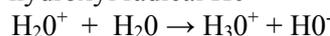
II. PHYSICAL AND BIOLOGICAL EFFECTS OF X-RAY

A. Physical Properties Of X-Rays

A radiation is defined as the transport of energy in space, which is then transferred to the matter. The radiation is quantified and measured in electron Volt (eV). According to the Electromagnetic Spectrum a radiation can be divided in Non-Ionizing Radiation (< 10 eV) or Ionizing Radiation (> 10 eV). When the radiation reaches the body, it excites the atoms of the molecules of biological tissues. Related to the absorbed dose, the biological consequences caused by ionizing radiation can change depending on the nature of radiation involved²²: α particle, β particle and X- Y Ray, where α and β are constituent of corpuscular radiation²³, while X and Y are electromagnetic radiation. Specifically, X-rays are classified as an electromagnetic, indirect ionizing radiation because it produces secondary electrons with high kinetic energies. These electrons in turn can cause damage in the absorbing matter. The electron vacancy in the atomic shell, caused by an ejection, is filled with an electron from an outer shell subsequently leading to the emission of a photon. A typical interaction between an X-ray photon and a water molecule is²⁴:



Where H_2O^+ is a highly reactive ion radical. The reaction between H_2O^+ and water molecule produces the hydroxyl radical HO^\cdot



Which is a highly reactive oxygen species and it is responsible for the biological effects of X-ray. ROS accumulation leads to apoptotic cell death²⁵ and is associated with the accumulation of damage that cannot be recovered in mitochondria and Nucleus. For example, typical features induced by radiation involve the Nucleus with induction of point mutations²⁶. However, when the apoptotic process does not eliminate a transformed cell, cytogenetic damages such as translocations pass to daughter cells²⁷. Thus, referring to the entire organism, radiation-induced DNA damages may lead to cancer or to hereditary diseases according to the specific cell damaged.

In general, X-ray biological effects are typically divided into two categories. The first category consists of exposure to high doses of radiation over short periods of time producing acute or short-term effects²⁸. The second category represents exposure to low doses of radiation over an extended period of time producing chronic or long-term effects. High doses tend to kill cells, while low doses tend to damage or change the functions of the several substructures. High doses can kill so many cells that tissues and organs are damaged or even destroyed²⁹. This in turn causes a rapid whole body response called the Acute Radiation Syndrome (ARS).

Low doses spread out over long periods of time and do not cause an immediate and clinical evident problem. The effects of low doses of radiation occur primarily at cellular level, and the effects may not be observed for many years. Here we will describe in details the biological effects of low and chronic exposure to X-ray.

B. Nuclear Effects Of X-Ray

There are three general categories of effects resulting from exposure to low doses of radiation. These are: 1) Genetic, when the effect is suffered by the offspring of the individual exposed. 2) Somatic³⁰, which primarily involves the individual exposed. Since cancer is the primary result, it is also called the Carcinogenic Effect. 3) In-Utero^{31, 32}, mistakenly considered as a genetic consequence of radiation exposure, because the effect, suffered by a developing embryo/fetus, is seen after birth.

In this contest DNA plays a key role in the response to radiation. Since the information on both strands of the DNA molecule is complementary, all injuries affecting only one side of the DNA double strand can be easily repaired by using the information on the intact strand as a template³³. Therefore, double strand breaks (DSB) are generally considered as the critical event for the induction of lethal lesions^{34, 35}. Mammalian cells are in general able to recognize and repair damage to DNA for a certain extent³⁶. The efficiency of these repair processes depends on the complexity of the damage induced³⁷. For example, single strand breaks can be easily repaired during the replication cycle, when the double strand has to be opened on one strand to allow the access of replication and reparative proteins to the DNA. But with increasing complexity, damage becomes more difficult to repair, and this might enhance the probability that this process cannot be accomplished correctly, leaving a partially repaired or modified DNA molecule. DNA damage can be induced by radiation in two different ways. On the one hand, radiation leads to ionizing events in the DNA molecule itself, subsequently leading to breakage of molecular bonds and disruption of one or both strands of the DNA. These events are termed 'direct effect'. On the other hand, radiation leads to the production of, highly reactive OH-radicals by radiolysis of the water molecules surrounding the DNA molecule. These radicals are able to migrate over distances of a few nanometers during their lifetime and are thus capable of damaging the DNA molecule of distant cells. This action is termed "indirect effect". However, these types of lesions do not necessarily occur separately, but instead, depending on the dose level, combinations of both effects can lead to DNA damage.

As mentioned above, X-ray are mainly indirectly ionizing because they do not directly induce chemical damage but produce secondary electrons with high kinetic energies such as hydrogen (H) and hydroxyls (OH). These fragments may recombine or may interact with other fragments or ions to form compounds, such as water, which would not harm the cell. However, they also combine to form toxic substances, such as hydrogen peroxide (H₂O₂), which can contribute to the destruction of the cellular structure, indicating that part of the toxic effects of X-ray are mediated by ROS.

C. Ros In The Intracellular Signaling: Only A Mediator Of X-Ray Induced Damage?

The perceived role of ROS in regulation of the cellular physiology has changed in the recent years. Indeed, if on a side they are considered as detrimental for cell survival, they also show important physiological roles and act as part of the intracellular signaling, promoting beneficial cellular processes as mitohermesis, defined as replacement and organization of the mitochondrial network. The list of intracellular targets of ROS is growing rapidly and several targets have been identified: 1) The mitogen-activated protein kinase (MAPK) family, also known as extracellular signal-regulated kinases (ERKs), is activated by exogenous and endogenous H₂O₂ in cells stimulated with growth factors³⁸. ERKs are important mediators of proliferation and their activation is implicated in vascular endothelial growth factor (VEGF)-mediated Endothelial Cell survival³⁹. 2) Akt/PKB kinase, is known to be involved in anti-apoptotic signaling and it is also regulated by ROS⁴⁰. Specifically, Akt is activated in the vascular endothelium through increased level of H₂O₂ by the membrane NAD(P)H oxidase which in turn is activated by shear stress. 3) Activation of the transcription factor NF- κ B, which is associated with Endothelial Cell dysfunction and vascular inflammation, is also regulated by ROS. These evidences suggest ROS as a specific cellular messenger able to promote either cellular survival and adaptation or apoptosis, according to the specific characteristics of the stressors. This knowledge may also change the view of ionizing radiation in clinic and therapy. The effects of ROS induced by radiation are different according to the dose and time of exposure^{41 42} and considering that the major ROS production is given by mitochondria⁴³, it would not be surprising that the cellular effects induced by X-ray depend on modification of mitochondrial function in a dose and time dependent manner⁴⁴. Indeed, recent works have uncovered that ROS directly participates to pivotal cell function like mitochondrial quality control⁴⁵⁻⁴⁷.

Mitochondria occupy a substantial portion of the cytoplasmic volume of eucaryotic cells, in particular cardiomyocytes. Each mitochondria is bounded by two highly specialized membranes, which have very different functions: 1) An outer membrane (O.M.) that contains many copies of transport protein called porin (MPTP)⁴⁸; 2) Inner

membranes (IM) that is usually highly convoluted, forming a series of unfolding, known as cristae, that project into the matrix. Together they create two separate mitochondrial compartments: the internal matrix (IM) and a narrower intermembrane space. In the mitochondria, the metabolism of sugars is completed, and the energy released is harnessed so efficiently that about 30 molecules of ATP are produced for each molecule of glucose oxidized. Oxidative phosphorylation is made possible by the close association of the electron carriers with protein molecules. The electron transport chain (ETC) resides on the inner membrane and it is composed by a series of protein complex that transfer electrons from electron donor to electron acceptors via redox reactions, and couples this electron transfer with the transfer of protons (H^+ ions) across the membrane. The proteins guide the electrons along the respiratory chain so that the electrons move sequentially from one enzyme complex to another without short circuits. Most importantly, the transfer of electrons is coupled to oriented H^+ uptake and release, as well as to allosteric changes in energy-converting protein pumps⁴⁹. The net result is the pumping of H^+ across the inner membrane from the matrix to the intermembrane space, driven by the energetically favorable flow of electrons. This movement of H^+ has two major consequences:

- Generates a pH gradient, and
- A voltage gradient, which is defined as the mitochondrial membrane potential.

A reduction of mitochondrial potential is an indicator of mitochondrial dysfunction and induces the mitochondria degradation and removal⁵⁰. This introduces the concept of "Mitochondrial dynamics"⁵¹ which refers to organelle fission, fusion, and subcellular translocation and this process is fundamental, in response to stress, to recover the mitochondrial damage and cellular survival⁵². In response to stress, a portion of damaged mitochondria loses its membrane potential and is cleaved from DRP-1 (Dynamin Related Protein)⁵³ generating two mitochondria daughters that are destined to elimination (mitophagy) or recovering (mitochondrial fusion). In response to the reduction of mitochondrial membrane potential and DRP-1 activation, the portion of injured mitochondria expresses PINK-1, a mitochondrial serine/threonine protein kinase⁵⁴. PINK-1 is intimately involved in "mitochondrial quality control" (QC) allowing

targeting mitochondria to elimination⁵⁰. Healthy mitochondria maintain a membrane potential and import PINK1 into the inner membrane where it is cleaved by PARL. Severely damaged mitochondria lack sufficient membrane potential to import PINK1, which then accumulates on the outer membrane. PINK-1 phosphorylates the ubiquitin and induces Parkin on Ser65 which is then allowed to move to mitochondria^{55, 56}. Parkin is a component of an E3 ubiquitin ligase complex, that together with other molecules such as P62, LC3-I/II, forms an ubiquitin-proteasome system designated to the removal and degradation of entire or part of mitochondria^{57, 58}. The mitochondria that has not lost its membrane potential activates the mitochondrial fusion^{59, 60} through mitofusin 1, mitofusin 2⁶¹⁻⁶³ and Opa-1⁶⁴ allowing recovering of the two "daughter" mitochondria (Fig.1).

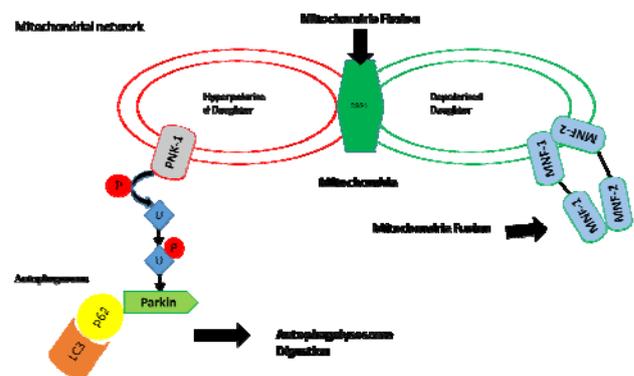


Figure 1: In response to stress a part of mitochondria loses its mitochondrial potential membranes and Drp-1 induces (mitochondrial fission), consequently PINK-1 is exposed on mitochondria damage and it drives mitochondria degradation through phosphorylation of ubiquitination and activation of ubiquitin ligase Parkin (autophagosome digestion). The mitochondria that has not lost its membrane potential activates the mitochondrial fusion through mitofusin 1, mitofusin 2 and Opa-1 with other part of mitochondria that has preserved its mitochondrial membrane potential to form a new functional mitochondria (Mitochondria fusion).

Together mitochondrial fusion and fission regulates the mitochondrial dynamics^{20, 65} and promote cellular survival in response to stress⁶⁶. In summary, the mitochondrial dynamics is implicated in structural remodeling of the mitochondrial network

and in homeostatic maintenance of mitochondrial DNA stability and respiratory function preventing or propagating programmed cell death, in particular in response to stress⁶⁷. The combined effects of continuous fusion and fission give rise to mitochondrial networks, to preserve organ functions.

In this context it is interesting to introduce the effect of X-ray exposure on the mitochondrial functions⁴³. X-ray exposition can directly damage mitochondrial membrane^{68, 69} and/or indirectly through increasing ROS production⁷⁰. In both cases, the consequence is the reduction of mitochondrial potential membranes and thus activation of fission/fusion process²⁰. As described above, these mechanisms are fundamental for cell survival and maintenance within physiologic conditions, therefore increased ROS production induced by X-ray can even promote the renewing of the mitochondria⁷¹. However, when the amount of accumulated ROS, as it happens with chronic exposure to low doses of X-ray, overcomes mechanisms of repair and it activates pro-apoptotic events and programmed cell death⁷².

II. X-RAY AND CARDIOVASCULAR EFFECTS: ROLE OF MITOCHONDRIA

Cardiovascular disease and cancer are the two leading causes of mortality and morbidity worldwide⁷³. These two pathological conditions frequently run together in presence of cancer disease treated with radiotherapy. Radiation therapy (RT) has evolved to be a cornerstone for treatment of various types of cancers (about 50% of patients with cancer are treated with radiotherapy). Chronic mediastina radiation can involve the pericardium, myocardium, valves and coronary vessels with pericardium being most frequently involved^{74, 75}. Thus, it is not surprising that for specific type of cancer like Hodgkin lymphoma (HL) an important cause of death is represented by cardiovascular complications^{76, 74, 77}. Several studies show that X-ray produce vascular endothelium abnormalities with resulting telangiectasia, thrombotic and inflammatory alteration⁷⁸ in large vessels, which can finally result in coronary and carotid artery disease^{79, 80}. The effects of X-ray are time and dose dependent and include a wide number of effects, starting from endothelial dysfunction, lipid and inflammatory cells infiltration till formation of atherosclerotic plaque^{81, 82}. In particular, in animal

model treated with elevated cholesterol-diet, the radiation stress abridges atherosclerosis, due to an increased macrophages infiltration into the arterial wall^{83, 84}.

The effects of X-ray on myocardium⁸⁵ is less known but it is emerging that this radiation can interfere with mitochondria functions, with relevant consequences considering the heart as a dynamic organ with an abundant number of mitochondria⁸⁶. Therefore, mitochondria damage can be pivotal in fostering the cardiac alterations produced by chronic irradiation⁸⁷.

Role of Mitochondria in mediating the effects of X-ray on cellular survival have been related in part to the direct effect on the external mitochondrial membrane and to the phenomenon of oxidative stress (Fig 2).

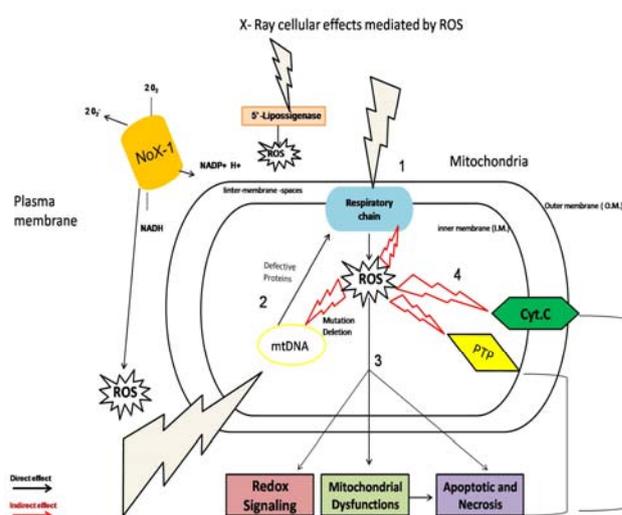


Fig 2: X-Ray cellular effects mediated by ROS. X-rays on plasma membrane induce an increase of ROS through activation of NOX1 and (5' lipossigenase). X-ray can target to mitochondria both directly and indirectly: through increased ROS production: 1) the compounds of respiratory chain. 2) mtDNA generating through mutation and deletion defective proteins to respiratory chain. 3) Alteration of redox signaling and mitochondrial dysfunctions that induces necrosis and apoptotic events. 4) Release of Cytochrome C outer mitochondria membranes into cytosolic fractions and formation of mPTP channel to induce respectively apoptotic and necrosis events.

However the specific molecular mechanisms are far to be elucidated. For future investigations we

may start from some observations: 1) The heart is an organ with an high energy demand, needed to accomplish its contractile function⁸⁸ 2) A preserved mitochondrial function is indeed fundamental for cardiac function and an extensive literature is now available showing the role of mitochondrial dysfunction in the setting of chronic diseases⁸⁹, like cardiac aging^{90,91}, ischemia or heart failure⁹²⁻⁹⁶. 3) X-ray used for treatment of LH can lead to a cardiac eccentric remodeling with systolic dysfunction, which is a phenotype observed in many other cardiac pathological conditions^{85, 95, 97}. Therefore the molecular mechanisms and mitochondrial process involved in myocardial injury induced by X-ray could not be so different to those observed in other cardiac diseases⁹⁸. It is possible speculate that X-ray chronic exposure as observed in patients treated with radiotherapy, the accumulate damage of mitochondria cannot be further recovered, as seen in human and animal model of cardiac aging and heart failure⁹⁹. Mitochondria are dynamic organelles where mechanism of mitogenesis and fission/fusion allows either produce new organelle or recover damaged mitochondria¹⁰⁰. However, it is also part of a network, with functional connection with endoplasmic reticulum¹⁰¹, nucleus and cytosol and it is exposed to the influence of several intracellular signaling that regulate mitochondria responses to the external stress. For example, the master regulator of mitochondrial biogenesis is PGC-1 α , which is not a resident mitochondrial protein¹⁰². This molecule lacks DNA-binding activity but interacts with and co-activates numerous transcription factors including NRFs on the promoter of mtTFA. PGC-1 α is enriched in tissue with high oxidative activity-like heart and brown adipose tissue and it is rapidly induced under conditions of increased energy demand and stress such as cold, exercise, and fasting. PGC-1 α can be regulated by different intracellular signaling (Thyroid hormone (TH), nitric oxide synthase (NOS/cGMP), p38 mitogen-activated protein kinase (p38MAPK), sirtuines (SIRT), calcineurin, calcium-calmodulin-activated kinases (CaMKs), adenosine-monophosphate-activated kinase (AMPK), cyclin-dependent kinases (CDKs), and β -adrenergic stimulation (β /cAMP) through post-translational modifications, rendering it as the integrative step for the different conditions^{103, 104}.

Mechanisms of fission/fusion are also governed by non-mitochondrial molecules and signalling. These pathways are activated in stress conditions,

such as H₂O₂, UV and ischemia, with following modifications of the mitochondrial morphology and dynamic^{105, 106}. We observed similar mitochondrial modifications in cells exposed to a single dose of X-ray irradiation, where the initial mitochondrial damage and disarrangements is there after recovered in a relative short period of time (about 8 hour), suggesting involvement of the mitochondrial quality control mechanism¹⁰⁷. Specific cytosol molecules could be triggered: for example Mitogen-activated protein (MAP) kinase cascade member extracellular-signal-regulated kinase (ERK) has been shown to phosphorylate the pro-fusion protein mitofusin (MFN) 1, modulating its participation in apoptosis and mitochondrial fusion¹⁰⁷. Moreover, ERK signalling can also indirectly modulates other proteins such as HSP90 and GRK2¹⁰⁸, which have been also demonstrated to localize at mitochondria^{109, 110}. GRK2 function in mitochondria is still debated and controversial, since it appears to act both as pro-death kinase and as protective in terms of improved biogenesis after ischemia-reperfusion injury¹¹¹. Our recent data, however, have shown that X-ray irradiation promotes GRK2 sub-cellular localization into mitochondria in a time dependent manner and GRK2 knockdown affects the mechanisms of mitochondrial recovering, thus suggesting a key role in the mechanism of quality control.

III. SUMMARY AND CONCLUSIONS

Hearts exposed to radiotherapy can accumulate mitochondrial damage that cannot be further recovered through the mechanisms of mitogenesis and/or quality control. The molecular mechanisms are probably not so distant from what observed in other conditions, where the reduced tolerance to stress is associated with impairment of pathways participating to the mitochondria-ER-nucleus network, fundamental to promote cellular adaptation to the stress. Models of heart failure post myocardial infarction, aging or diabetes¹¹² have shown the central role of mitochondrial dysfunction in the progression of the disease and the related discoveries are leading to specific approaches aimed to recover mitochondrial dynamic and functions¹⁰⁷. Therefore, a new challenge for the years to come will be the identification of the specific role played by this organelle in cardiac alterations induced by X-ray stress and so the development of new approaches to

enhance and/or preserve mitochondrial functions and network during chronic radiation stress.

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