New challenges to promote a deeper interaction between e-learning and research

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ABSTRACT. With the aim to illustrate to the students what really happens in a radiobiology's laboratory, a virtual lab was created. Through an interactive simulation of both X-ray generator and mouse models, the students can expand their knowledge about the biological effects induced by ionizing radiation using new pedagogical and technological strategies. The realized multimedia product has also been inserted as a case study to promote a deeper interaction between e-learning and research. The carcinogenic effects of ionizing radiation are induced by a direct irradiation of DNA molecule, but also by not direct cells and tissues exposure (so called: Abscopal effects). Ionizing radiation exposure can also induce non-cancer effects. In this context, genetically engineered radiation-susceptible mouse models represent a powerful tool to improve both understanding of radiation effects and assessment of cancer and non-cancer risks from ionizing radiation exposure. Our research field is focused on the study of high-impact human health-related pathologies such as: Medulloblastoma, Basal Cell Carcinoma, Lens opacities and Cardiovascular disease.

KEYWORDS: *Abscopal effect, Cancer effect, Interactive teaching/learning, Ionizing radiation, Non cancer effect, Radiobiology, Simulation, Virtual labs*

Introduction

In an era with rapid technological advancements, new methods of education need to be developed to augment the traditional teaching paradigms. The distance learning represents the optimal solution to overcome classical teaching without being in regular face-to-face contact. Because most conventional teaching materials are linear and static, in the context of e-learning new modern computer softwares

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offer the possibility to upload links or videos of virtual laboratories to the lessons making them more dynamic. Providing students with an engaging education with electronic media is very important in particular for scientific courses that need to be enriched and integrated with a teaching complex dynamic system using rich-media animations, real-time simulations, and virtual laboratories. These new strategies and tools are an excellent occasion for students to enrich their own knowledge and to improve their profile and skills. The idea to produce a specific radiobiology virtual lab for the Marconi University's Biology course stems from the need to strengthen the deeper interaction between e-learning and research that can contribute to better professional development of students.

Radiobiology

Radiobiology is a branch of science combining two disciplines, radiation physics and biology, the purpose of which is to study the action of ionizing radiation on living things. Ionizing radiation is a type of energy released by atoms that travels in the form of electromagnetic waves (gamma or X-rays) or particles (neutrons, beta or alpha). Ionizing radiation carries sufficient energy to be dangerous to living cells an when an organism is exposed to radiation, the amount of damage it suffers will depend on the energy carried by the radiation (Hall, 1994).

Today, exposure of the population to natural radiation is to some extent unavoidable. Medical exposure of patients during diagnosis and therapy, and of population groups during screening, is now an indispensable part of modern medicine. The exposure of workers, and to a smaller extent of the public, to low levels of radiation from nuclear energy production and other industrial uses of ionizing radiation have become an integral part of industrialized society. In this context it is essential to understand the mode of interaction of radiation with biological matter. In fact, the main purpose of radiobiological research activities are centered on questions relating to biological effects from different types of radiation and their dose dependence, the biological processes in cells/tissues that mediate the health effects of low dose radiation (both cancer and non-cancer effects), the individual variability and direct assessment of health effects through epidemiological study of groups exposed to low doses.

In order to achieve the aforesaid purposes, scientific and technical support was provided by the Technical Unit of Radiation Biology and Human Health in ENEA Casaccia, Rome, Italy, and in particular the Laboratory of Radiation Biology and Biomedicine.

This laboratory has been working in radiobiological research for many decades, and is positioned as a leader in this field and one of the most important groups in Europe working in animal radiobiology with a view to understand the mechanisms and genetics of radiation-induced biological effects. The Marconi University expressed interest in this research field by a multiyear scientific collaboration between its Department of Nuclear Sub-nuclear and Radiation Physics and the Laboratory of Radiation Biology and Biomedicine of ENEA.

Mechanisms of action of ionizing radiation on the biological structures

Target theory

When cells are exposed to ionizing radiation the standard physical effects between radiation and the atoms or molecules of the cells occur first, and the possible biological damage to cell functions follows later. The biological effects of radiation result mainly from damage to the DNA, which is the most critical target within the cell; however, there are also other sites in the cell that, when damaged, may lead to cell death. When directly ionizing radiation is absorbed in biological material, the damage to the cell may occur in one of two ways: direct or indirect. In the direct action, radiation interacts directly with the critical target in the cell. In the indirect action, radiation interacts with other molecules and atoms (mainly water, since about 80% of a cell is composed of water) within the cell to produce free radicals, which can, through diffusion in the cell, damage the critical target within the cell.

Non-target theory

A range of evidence has now emerged that challenges the classical effects resulting from targeted damage to DNA. These effects have also been termed "non-(DNA)-targeted" (Ward, 1999) and include radiation-induced bystander effects, genomic instability, adaptive response, low dose hyper-radiosensitivity, delayed reproductive death and induction of genes by radiation. An essential feature of "non-targeted" effects is that they do not require a direct nuclear exposure by irradiation to be expressed and they are particularly significant at low doses. This new evidence suggests a new paradigm for radiation biology that challenges the universality of target theory. The radiation-induced bystander effect is a phenomenon whereby cellular damage such as sister chromatid exchanges (Nagasawa, Little, 1992), chromosome aberrations, apoptosis, micronucleation, transformation, mutations and changes in gene expression (Azzam et al., 2001) is expressed in unirradiated neighbouring cells near to an irradiated cell or cells due to cell-to-cell communication or soluble factors released by irradiated cells. Interactions between hit and non-hit cells after exposure to ionising radiation have been known for many years in radiation biology and the vast majority of these effects are described in cell-culture systems, while in vivo validation and assessment of biological consequences within an organism remain uncertain. In the last few years, a large number of papers were published demonstrating evidence for the radiation induced non-targeted (abscopal) effects *in vivo* and demonstrating that bystander effects are factual in vivo events with carcinogenic potential, and implicate the need for re-evaluation of approaches currently used to estimate radiation-associated health risks (Mancuso et al., 2008).

Radiation effects on the biological matter: cancer and non-cancer effects

The fact that ionizing radiation causes cancer in humans has been known for over a century. In 1902, the first radiation-induced cancer had been reported in an area of ulcerated skin. By 1911, there were also reports of leukaemia arising in radiation workers (Upton, 1986). Our understanding of radiation carcinogenesis has vastly progressed since the Second World War because of the important epidemiological evidence arising from the Life Span Study of the Japanese Atomic Bomb survivor cohort that is absolutely crucial to our understanding and estimation of cancer risk from ionising radiation.

In addition to cancer, ionizing radiation exposure can induce non-cancer effects. Analyses of the Life

Span Study mortality data (1950-1997) show a statistically significant dose-response pattern for death from diseases other than cancer (Shah et al., 2012).
These effects occur when cell death is so extensive to cause functional impairment of the tissue or organ. The main non-cancer effects are cardiovascular disease, neurocognitive effects and lens opacities. It has been traditionally assumed that health effects other than cancer show a threshold at doses that are well above the levels of exposures typically encountered in the public environment, at work, or from medical uses of ionizing radiation. The mechanisms responsible for the non-carcinogenic effects of radiation are for the most part unknown (Preston et al., 2003).

In vivo Radiobiology

In vivo Radiobiology investigates the biological processes and the molecular mechanisms through which ionizing radiation causes health effects, using animal models.
The use of animals in research is essential for development of new and more effective methods for diagnosing and treating diseases that affect both humans and animals. Scientists use animals to learn more about health problems, and to assure the safety of new medical treatments. Medical researchers need to understand health problems before they can develop ways to treat them. Some diseases and health problems involve processes that can only be studied in living organisms.
Animals are biologically similar to humans and their genome can be easily manipulated and analyzed (mice share more than 98% DNA similarity humans). Therefore, animals are susceptible to many of the same health problems as humans and the transgenic methodology affords a tool for the study of some human diseases. Animals have a shorter life cycle than humans and as a result, they can be studied throughout their whole life span or across several generations. In addition, scientists can easily control the environment around animals (diet, temperature, lighting), which would be difficult to do with humans. To understand biological mechanisms underlying radiation-related cancer, non-cancer and abscopal effects, animal models represent a useful tool.

From our own experience

ENEA has in house unique infrastructures in support of *in vivo* radiobiology activities. The infrastructures available include X-ray generators and the animal house where many mouse models are maintained. To provide quantitative and mechanistic information on the biology of ionizing radiation responses, two genetically engineered mouse models are especially used: the Patched1 heterozygous knockout (Ptc1+/-) and the Apolipoprotein E knock out (ApoE-/-) mice.
The Ptc1+/- mice have many features of Gorlin syndrome, a rare hereditary condition predisposing to spontaneous tumorigenesis. In this mouse model ionizing radiation synergizes with Ptc1 mutation to induce multiorgan tumorigenesis and dramatically accelerates tumor development. This model was useful to understand the molecular mechanisms controlling the development of basal cell carcinoma (BCC) and medulloblastoma (Mb) (Mancuso et al., 2004; Pazzaglia et al., 2004). Moreover, the extreme radiosentivity of these mice was strategic to also investigate non-cancer pathologies such as the lens opacity (De Stefano et al., 2015).
ApoE deficient mice have been critical for elucidation of factors affecting atherogenesis and in

fact they show impaired clearing of plasma lipoproteins and develop spontaneous atherosclerotic lesions resembling those seen in humans. As a consequence, they represent an ideal mouse model to establish, *in vivo*, the atherogenic process (Kolovou et al., 2008).

Cancer effects

Medulloblastoma is the most common primary central nervous system (CNS) tumor in children (Figure 1), arising presumably by transformation of granule neuron precursor (GNP) cells, and represents about 20% of all pediatric CNS tumors (Pazzaglia et al., 2004).

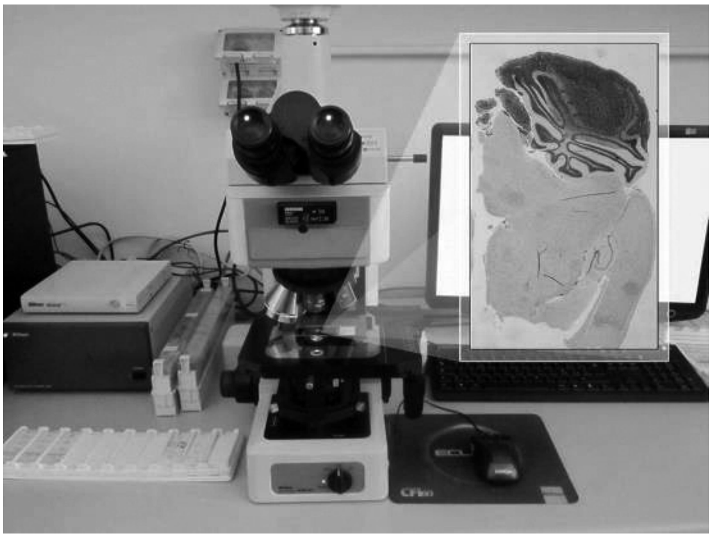

Figure 1. Microscopic image: Medulloblastoma stained with Hematoxylin and Eosin

Basal cell carcinoma is the most common skin cancer, accounting for approximately 70% of all skin malignancies (Figure 2) (Mancuso et al., 2004). Its incidence is increasing worldwide. Risk factors for BCC formation are exposure to ultraviolet or ionizing radiation, immunosuppression, or a genetic predisposition (Kwasniak, Garcia-Zuazaga, 2011).

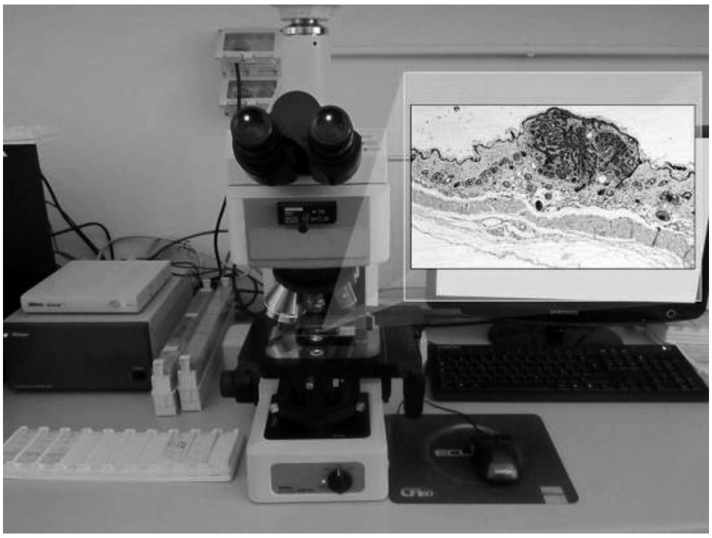

Figure 2. Microscopic image: Basal cell carcinoma stained with Hematoxylin and Eosin

Non-cancer effects

Cataract (the opacification of the ocular lens) is the most frequent cause of blindness worldwide.The eye is well known as one of the most radiosensitive tissues of the body and it is clearly recognized that cataracts can be induced by ionizing radiation exposure (Figure 3) (De Stefano et al., 2015).

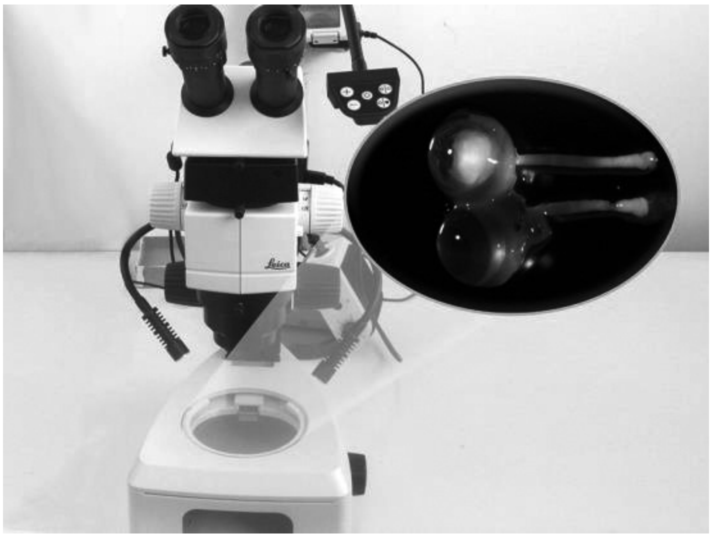

Figure 3. Tridimensional image: cataract and normal eyes

Atherosclerosis is a multifactorial disease, resulting from interactions between genetic and environmental factors, and susceptible to modification by radiation exposure (Singh et al., 2002). It is an inflammatory disease of the arteries (Figure 4) that can lead to ischemia of the heart and brain, resulting in infarction. Epidemiological evidence has established links between cardiovascular disease and exposure of the heart and major vessels to doses above 0.5 Gy.

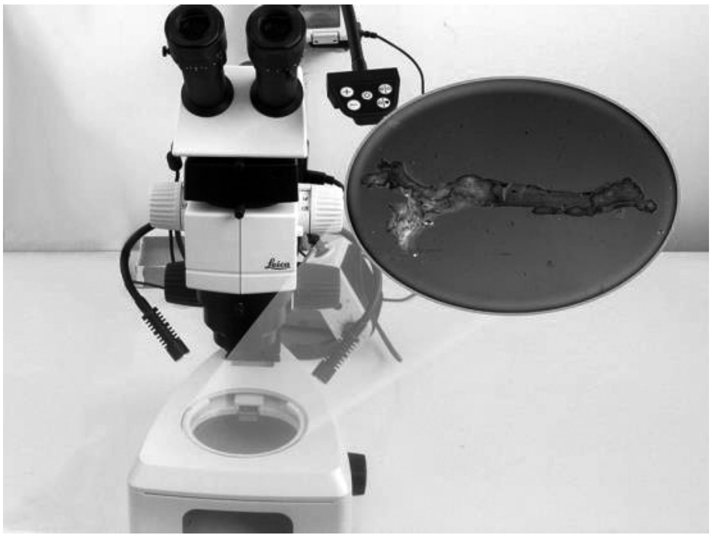

Figure 4. Tridimensional image: aortic plaques stained with Oil-red-O

The virtual laboratory

The multimedia product proposed, designed and based on our personal experience, has the aim to become a tool to improve the knowledge and skills of students in the field of radiobiological research.
The first step in an experimental study of Radiobiology is to know what effects induced by ionizing radiation on health we want to investigate.
The second step is to find *a way* to make it.
In this virtual lab, the students can answer to above questions simulating an irradiation setup, using a X-rays generator and an appropriate mouse model.
Each simulation of irradiation is named "level of irradiation".

Level 1: is shown a Ptc1+/- mouse, at neonatal age, whole body exposed to 3Gy of X-ray. An interactive excel file containing mathematical calculations, allows to check the final dose of delivered X-rays. The consequences of this exposure on different tissues can be visualized clicking on flashing dots drawn on the mouse that appears in the screenshot after the irradiation period (Mb, BCC and cataract) (Figure 5a).

Level 2: is shown an ApoE-/- mice, at adult age, whole body exposed to 6Gy of X-ray. After the simulation of irradiation it is possible to see and analyze cardiovascular effects. Clicking on flashing dots drawn on the mouse, students can visualize the details of the specific pathology (atherosclerotic plaques) (Figure 5b).

Level 3: is shown a Ptc1+/- mouse, at neonatal age, shielded irradiated with 10Gy of X-ray. Heads are protected by individual custom-built lead cylinders that allow to exposure only the lower part of the body. Clicking on flashing dots drawn on the mouse, you can see the appearance of the disease in shielded tissues (Mb, BCC) (Figure 5 c).

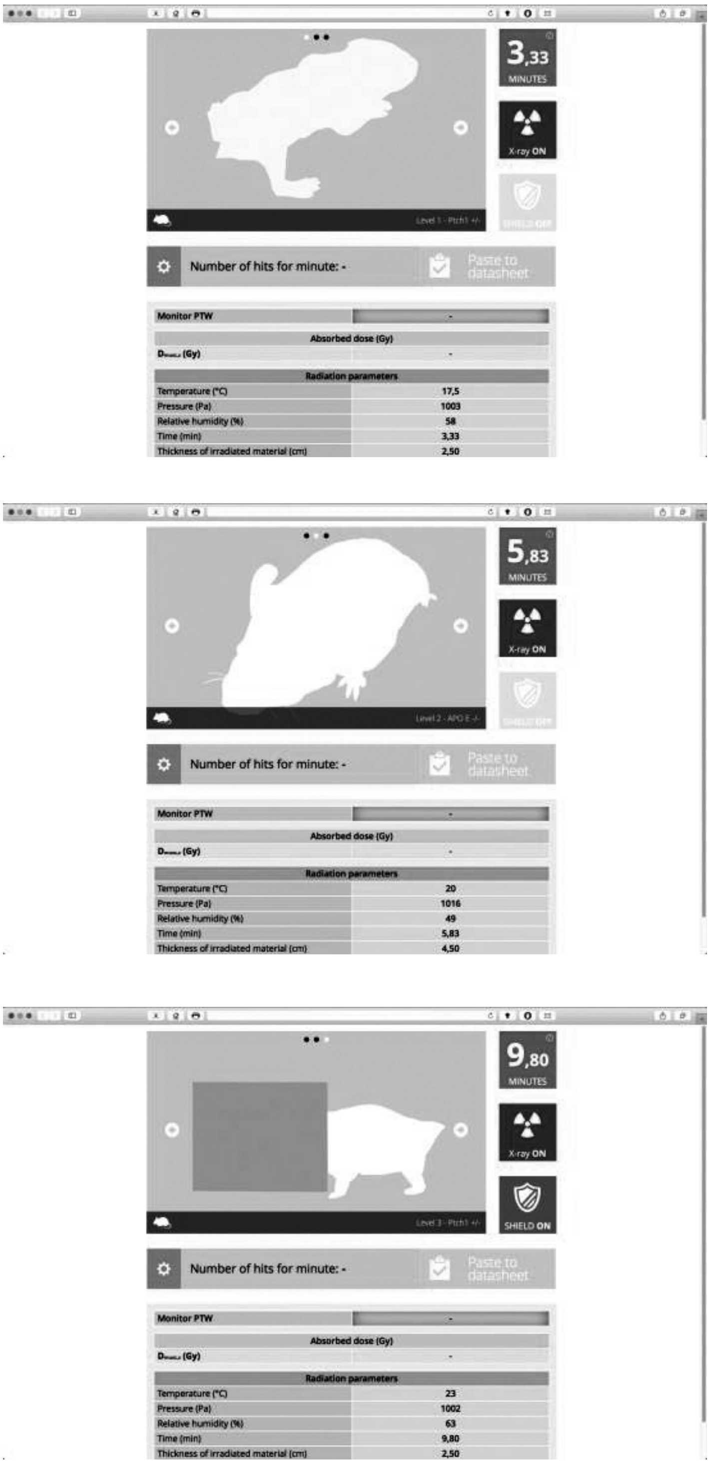

Figure 5. The three levels of RX exposure (a, b, c)

Conclusion

A virtual laboratory has been created to allow a deeper awareness on research in biomedicine field and to offer and promote a greater scientific knowledge.
This virtual lab will be inserted as a case study in the course Applied Biology and in the future could be improved with additional functions to give more information to make the students protagonist and capable of planning an experiment through the use of various laboratory techniques. The goal is to make practical what you learn in theory and to make teaching/learning more dynamic using the new technologies.

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Sintesi

In un'epoca di rapidi progressi tecnologici, i tradizionali paradigmi di insegnamento vengono man mano sostituiti da nuovi metodi di formazione, all'interno di un nuovo progetto educativo basato sull'insegnamento a distanza. Questa nuova tecnica di formazione, utilizzando materiali didattici che si avvalgono dell'uso delle nuove tecnologie, informatiche e telematiche, offre la possibilità di rendere le lezioni più dinamiche e interattive. In particolare, l'uso di animazioni multimediali, simulazioni in tempo reale e laboratori virtuali risultano indispensabili per l'insegnamento a distanza di corsi scientifici, in quanto arricchiscono il patrimonio di informazioni e di conoscenza degli studenti e le loro competenze.

In questo contesto, con l'obiettivo di illustrare agli studenti ciò che realmente accade in un laboratorio di radiobiologia, è stato creato un laboratorio virtuale, con lo scopo di mettere in pratica ciò che viene appreso teoricamente e per rendere l'insegnamento e l'apprendimento più dinamici. Agli studenti viene fornita una parte teorica a supporto del prodotto multimediale che rappresenta la parte pratica.

La parte teorica introduce i concetti base della radiobiologia, approfondendo sui meccanismi di azione delle radiazioni ionizzanti sulla materia biologica e sui meccanismi molecolari attraverso i quali le radiazioni ionizzanti provocano effetti sulla salute (effetti cancerogeni e non cancerogeni) e descrivendo l'importanza della sperimentazione sugli animali che forniscono modelli per lo studio di patologie ad alto impatto sulla salute umana.

La parte pratica consiste in una simulazione interattiva dell'uso di un generatore di raggi X e di un modello animale radiosensibile. In particolare, il prodotto multimediale proposto, progettato sulla base delle attività di ricerca che si svolgono presso i laboratori di Biologia e Biomedicina dell'ENEA, ha l'obiettivo di essere utilizzato come strumento per migliorare le conoscenze e le competenze nel campo della ricerca radiobiologica. Utilizzando il prodotto multimedia, lo studente diventa autonomo e ha la possibilità di scegliere l'animale geneticamente modificato che vuole utilizzare, in base al tipo di effetto indotto dalle radiazioni ionizzanti che vuole indagare (medulloblastoma, carcinoma a cellule basali, opacità della lente e malattie cardiovascolari) e la modalità per indurre quell'effetto, selezionando diversi livelli di irraggiamento.

Il laboratorio virtuale è stato creato per consentire una più profonda consapevolezza della ricerca in campo biomedico e per offrire e promuovere una maggiore conoscenza scientifica. Questo prodotto multimediale verrà inserito come caso di studio nel corso Biologia Applicata e rappresenta un primo esempio di interazione tra e-learning e ricerca, fornendo agli studenti la possibilità di progettare un esperimento attraverso l'uso di varie tecniche di laboratorio.