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Investigations of DNA damage induction and repair resulting from cellular exposure to high dose-rate pulsed proton beams

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Abstract. Studies regarding the radiobiological effects of low dose radiation, microbeam irradiation services have been developed in the world and today laser acceleration of protons and heavy ions may be used in radiation therapy. The application of different facilities is essential for studying bystander effects and relating signalling phenomena in different cells or tissues. In particular the use of ion beams results advantageous in cancer radiotherapy compared to more commonly used X-rays, since the ability of ions in delivering lethal amount of doses into the target tumour avoiding or limiting damage to the contiguous healthy tissues.
At the INFN-LNS in Catania, a multidisciplinary radiobiology group is strategically structured aimed to develop radiobiological research, finalised to therapeutic applications, compatible with the use of high dose laser-driven ion beams. The characteristic non-continuous dose rates with several orders of magnitude of laser-driven ion beams makes this facility very interesting in the cellular systems' response to ultra-high dose rates with non-conventional pulse time intervals cellular studies. Our group have projected to examine the effect of high dose laser-driven ion beams on two cellular types: foetal fibroblasts (normal control cells) and DU145 (prostate cancer cells), studying the modulation of some different bio-molecular parameters, in particular cell proliferation and viability, DNA damage, redox cellular status, morphological alterations of both the cytoskeleton components and some cell organelles and the possible presence of apoptotic or necrotic cell death.
Our group performed preliminary experiments with high energy (60 MeV), dose rate of 10 Gy/min, doses of 1, 2, 3 Gy and LET 1 keV/μm on human foetal fibroblasts (control cells). We observed that cell viability was not influenced by the characteristics of the beam, the irradiation conditions or the analysis time. Conversely, DNA damage was present at time 0, immediately following irradiation in a dose-dependent manner. The analysis of repair capability showed that the cells irradiated with 1 and 2 Gy almost completely recovered from the damage, but not, however, 3 Gy treated cells in which DNA damage was not recovered.
In addition, the results indicate the importance of the use of an appropriate control in radiobiological in vitro analysis.

INTRODUCTION

Radiobiology, as "the science behind radiotherapy", studies the effects of radiation on biological tissue using radiobiological insights gained to improve the efficacy of radiation treatments. An understanding of radiobiology is essential in making use of the possibilities that new technology gives us. It is necessary to start using models for predicting treatment outcomes as part of the treatment planning process. Usually, the optimal radiobiology research project and the analysis of results gained require the sharing of expertise and experiences among medical physicists, radiobiologists and clinical oncologists.

Up to now, X-rays in the low MeV energy range represent the majority of ionising radiations used in the treatment of several patients worldwide with cancerous tumours. Since the invention of cancer radiotherapy, its primary goal has been to maximise lethal radiation doses to the tumour volume while keeping the dose to surrounding healthy tissue at zero. Higher quality, very high Energy (VHE) particle beams, such as those produced by a laser plasma accelerator, could be used in radiotherapy and hadrontherapy, and could also provide better clinical results. However, while hadrontherapy is less invasive than traditional radiotherapy, and continues to be indicated for a greater number of tumour types, its use cannot completely avoid damage to surrounding healthy tissue.

At the INFN-LNS in Catania, a multidisciplinary radiobiology group is strategically structured with the intention of developing and exchanging both knowledge and skills in order to obtain scientific information useful for the transfer of results to clinical applications. The group includes researchers working at the

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University of Catania, the University of Naples Federico II, the INFN-LNS in Catania and Queen's University (UK).

The main objective of our studies is to establish a procedure for cell handling and irradiation conditions for radiobiological research compatible with the use of high dose laser-driven ion beams. The therapeutic applications of laser-driven ion beams may consist of non-continuous dose rates with several orders of magnitude higher than those normally used. This feature makes these beams very interesting, especially in the study of the cellular systems' response to ultra-high dose rates with non-conventional pulse time intervals. Dose-rate effects are well documented when irradiation regimes typically at Gy/min are lowered towards chronic exposure conditions (i.e. down to Gy/s), but very little is currently known about effects at much higher rates. So far, experimental results have been limited to those of low energy (up to 20 MeV) proton beams or IORT electron beams. The biological response of the cellular systems to higher-rate irradiation conditions needs to be explored largely in view of their clinical use.

In ELIMED project, our intention is to study some aspects of the bio-molecular responses of different cellular systems (tumour and non-tumour cells) irradiated at high dose rates and with pulsed ion beams.

From the available literature data, it is well known that some of the main desirable outcomes of irradiation treatment are the proliferation arrest of a cancer cell and, subsequently, the induction of cell death by apoptosis or necrosis through activation of different mechanisms, such as: structural/functional DNA alterations, the cellular homeostasis destabilisation, including the modulation of redox state and the disintegration of the cytoskeleton.

A number of cell functions occur in irradiated cells with ionizing radiations, following the physical interaction between the radiation and the cellular molecules. They are particularly evident at DNA level, which is the main target within the cell (1). Damaging events, when they occur, are mostly immediate and are due to metabolic alterations as a consequence of radiation on enzyme systems or cytoplasmic organelles and/or DNA. Biological damage can be influenced by both physical factors, which in this case is named relative biological effectiveness (RBE) - a parameter that is influenced by different factors linked to the characteristics of the radiation, temperature, exposition time and to linear energy transfer (LET - Linear Energy Transfer); and biological factors, such as cell and tissue type and oxygen levels on cell cycle phase and DNA repair capability (Fig.1). Among the biological elements that may influence the cell ability to repair and its sensitivity to radiation, a key factor is the phase of the cell cycle in which cells are found at the time of irradiation, G1, S, G2 or M. (2)

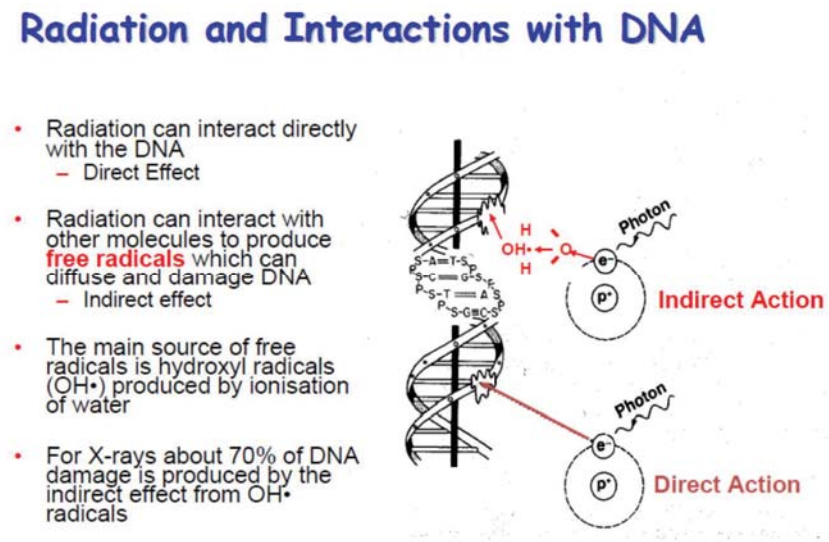

FIGURE 1 Some radiation elicited damages on DNA

METHODS

In order to study the effects elicited by high dose rate and pulsed beams, the research group working at the University of Catania will study the biologic effects on both foetal fibroblasts (normal control cells) and DU145 (prostate cancer cells) by employing the following radiobiological tests.

First method: Clonogenic test and MTT assay. These assays will be performed to determine the relative biological effectiveness (RBE) of these unconventional ion beams on the cellular proliferation/viability. The Clonogenic test studies cell proliferation (3). Cells are plated in Petri dishes and incubated until each surviving cell (*clonagen)* has produced a macroscopic colony, then the colonies on each plate counted. For each dose, a surviving fraction, S, may be calculated as the number of colonies of treated cells normalized respect to the control untreated cells. MTT is a colorimetric assay usually used for either proliferation or complement-mediated cytotoxicity assays (Fig.2). It is used to measure viable cells in respect to mitochondrial functionality. MTT is a pale yellow substrate that is metabolised by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not metabolise significant amounts of MTT (4).

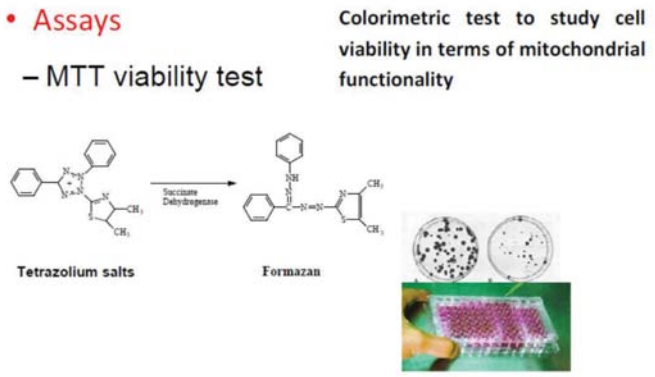

FIGURE 2. MTT assay

Second method: Different version of the comet assay (alkaline, neutral, Fpg enzyme, repair). This is a gel microelectrophoresis method, based on the technique developed by Ostling and Johanson in 1984 (5), for measuring a variety of types of DNA damage in individual cells, DNA breaks (SSBs and DSs), DNA alkali labile sites, crosslinks, DNA bases damage (8 OH guanosine DNA damage by Fpg enzyme use), repair capability of damage and apoptotic cells (6) (Fig.3).

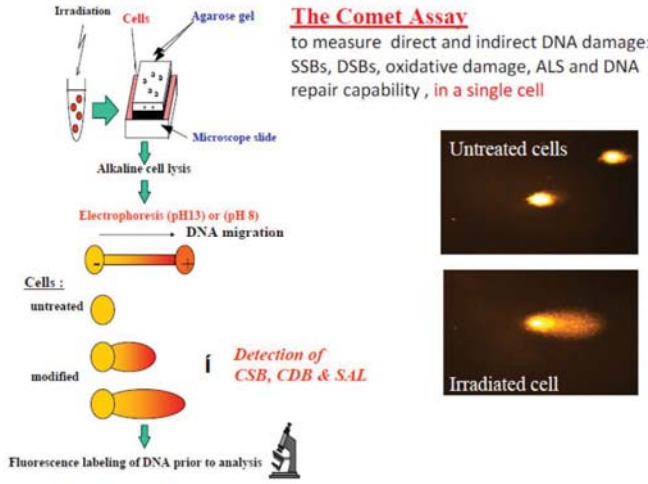

FIGURE.3. Comet assay method

The use of the comet assay in radiobiological research includes the analysis of inter-individual differences in response to radiation, the effects elicited by the oxidative stress promoted by the radiation and the effects of charged particles on DNA fragmentation patterns. Cells are embedded in agarose, lysed, subjected to an electric field, stained with a fluorescent DNA-binding stain, and viewed using a fluorescence microscope. Fragmented DNA migrates further in the electric field, and the cell then resembles a "comet" with a bright fluorescent head and a tail region, which increases in length and fluorescence proportionally to the damage. The damage may be quantified by video imaging analysis to define appropriate "features" of the comets.

Third method: The cellular redox state has important effects on the control of cell survival, apoptosis and/or the expression of tumor suppressor genes. Redoxomic analysis, aimed at exploring the effects of unconventional ion beams on the redox cellular status (ROS level and antioxidant enzymes) and the expression of some of the proteins involved in radioresistence and radiosensibility, by the use of the 2D electrophoresis proteomic approach (7); The Redoxomica is a new branch of applied biochemistry and molecular diagnostics having as objectives: a) to evaluate the production of radical species (ROS/RNS) and the activity of antioxidant systems in a living organism; b) to identify interactions between oxidants and antioxidants in a cell a tissue, a body, under basal condition as well as in response to radiation (Fig.4).

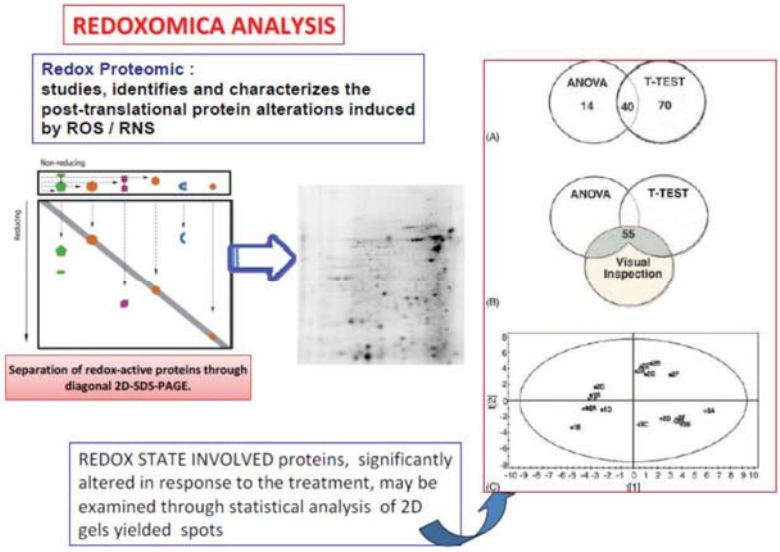

FIGURE.4. Schematic demonstration of Redoxomic analysis

Fourth method: Cell death analysis in order to differentiate between apoptotic and necrotic cell death. When DNA is damaged and not successfully repaired, the cell may die – cell death may occur (interphase death) or during its attempt to divide (mitotic or reproductive death) or after a few cell divisions (abortive colonies). Apoptosis is an active, naturally occurring process of programmed cell death – biochemical pathways within a cell which lead to its own organized dismantling. In contrast, necrosis is the premature death of cells and living tissue, caused by external factors, with membrane disruption, respiratory poisons and hypoxia which cause ATP depletion, metabolic collapse, cell swelling and rupture leading to inflammation.

5 Electronic microscope analysis (SEM and TEM), in order to observe morphological alterations of both the cytoskeleton components and some cell organelles, but also the presence/absence of apoptotic cells (8) (Fig.5).

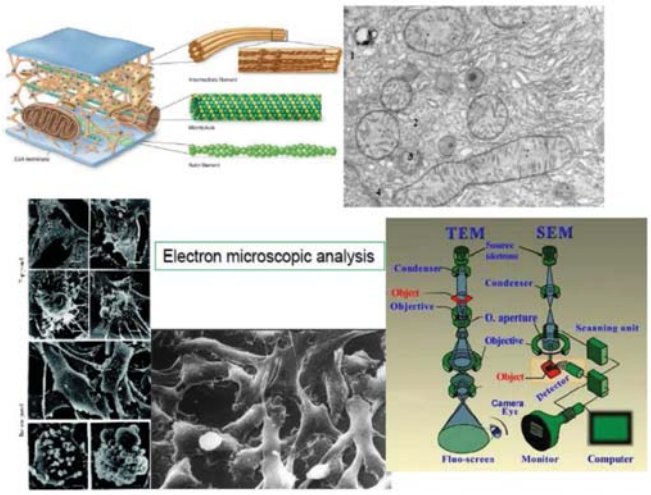

FIGURE. 5. Schematic demonstration of SEM and TEM analysis

PRELIMINARY RESULTS

This radiobiological research will be first implemented using the maximum dose rate and pulse frequency of conventional accelerators (i.e., cyclotron at the INFN-LNS) and available laser sources (i.e., TARANIS laser at QUB) with the aim of developing protocols, experimental routines and solid data sets for high power laser studies.

Our group performed preliminary experiments (MTT assay, alkaline COMET ASSAY and DNA repair capability at time 0, and after 45 min at 37°C) in INFN-LSN Catania with high energy (60MeV), dose rate of 10Gy/min, doses of 1, 2, 3 Gy and LET 1keV/µm on human foetal fibroblasts (control cells). As control cells, we utilised a negative control (cells maintained in incubator at 37°C) and a positive control cell maintained in the CATANA room close to the cells being irradiated.

We observed that cell viability was not influenced by the characteristics of the beam, the irradiation conditions or the analysis time. Conversely, DNA damage was present at time 0, immediately following irradiation in a dose-dependent manner. The analysis of repair capability showed that the cells irradiated with 1 and 2 Gy almost completely recovered from the damage, but not, however, 3Gy treated cells in which DNA damage was not recovered.

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REFERENCES

1. Steel, G.G., *Basic Clinical Radiobiology*, Arnold, 2002
2. P.A. Jeggo,M. Lobrich, *Contribution of DNA repair and cell cycle checkpoint arrest to the maintenance of genomic stability*, **5**: 1192-1198 (2006).
3. Potter MD, Suchowerska N, Rizvi S, McKenzie D. *Australas Phys Eng Sci Med.* **34**:345-350 (2011).
4. Cardile V, Bellia M, Lombardo L, Scifo C, Renis M. *Oncol Rep.***14**:981-985 (2005).
5. Ostling O, Johanson KJ. *Biochem Biophys Res Commun* **123**:291-298 (1984).
6. Renis M, Calandra L, Scifo C, Tomasello B, Cardile V, Vanella L, Bei R, La Fauci L, Galvano F.*Br J Nutr* **100**:27-35 (2008**)**.
7. Lin ST, Lo YW, Chang SJ, Wang WC, Chang MD, Lyu PC, Chen YW, Chou HC, Chan HL. *J Pharm Biomed Anal*. **78**-79:1-8 (2013).
8. Scifo C, Milasi A, Guarnera A, Sinatra F, Renis M. *Oncol Res.* **15**:409-21 (2006).